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STUDIES ON THE ACTION OF OIL IN THE CONTROL OF MYCOSPHAERELLA MUSICOLA ON BANANA LEAVES.[1]

L. Calpouzos, T. Theis, Carmen M. Rivera, and C. Colberg[2]

SUMMARY

The antifungal action of petroleum and silicone oil sprays, which control the Sigatoka leaf-spot disease of bananas, caused by *Mycosphaerella musicola* (*Cercospora musae*), was studied. The materials tested did not significantly inhibit conidial or ascospore germination; stomatal penetration was not sufficiently inhibited to account for disease control. They did not inhibit mycelial growth in culture flasks, or impair ascospore discharge or conidial production on diseased leaves. They possibly inhibit the fungus inside the leaf at some stage after stomatal penetration and before symptom appearance.

Oil sprays are replacing Bordeaux mixture in controlling an important fungus leaf-spot disease of banana[3] (3). This change stems from the work of Guyot and Cuille (5, 6), who found that petroleum spray oils without a fungicide gave excellent control of *Mycosphaerella musicola* Leach (*Cercospora musae* Zimm.), which causes the Sigatoka leaf-spot disease. The oil is applied in pure form as a mist at the rate of 1–3 gal./A. This is the first modern, large-scale use of oil to control a fungus disease.

Information on the effect of oils on fungi is limited. A few reports (1, 7, 8, 9) state that various oils are able to control certain fungus diseases. Others, however, describe the use of oil to preserve viable cultures of fungi (2) or to act as a carrier for fungus spores for inoculation (10). Although oil sprays are widely used for insect control, their use in disease control is not recognized. There are no data to indicate the mechanism by which oil can control a fungus disease.

The increasing importance of oil sprays for controlling the Sigatoka disease suggested that their action should be investigated. This paper deals with the effect of selected petroleum and silicone oils on the germination, penetration, vegetative growth, ascospore discharge, and conidial sporulation of *M. musicola*.

MATERIALS AND METHODS.—One or more of the following materials were used in each of the experiments: Orchard Spray Oil No. 35[4] (viscosity 72.6 S.S.U. at 100°F, unsulfonated residue 84–85%), Orchard Spray Oil No. 40[4] (viscosity 108 S.S.U. at 100°F, unsulfonated residue 84–85%), pharmaceutical grade mineral oil[5] (viscosity 365 S.S.U. at 100°F, unsulfonated residue 98–100%), Silicone Fluid 555[6] (viscosity 82.5 S.S.U. at 100°F, unsulfonated residue analysis not applicable), and Bordeaux mixture 5-5-50, the traditional spray for Sigatoka control. Orchard Spray Oil Nos. 35 and 40 have been applied in banana plantations, but pharmaceutical mineral oil and Silicone 555 have not, because they are too expensive. The latter 2 materials were included in certain of these experiments, since they differ chemically from either oil No. 35 or 40. The oils were stored at room temperature in closed metal containers during the testing period. The Bordeaux mixture was prepared fresh before each application. In experiments involving leaf material, unless noted otherwise, the treatment was applied to the upper surface of young Gros Michel banana leaves. The leaves were sprayed to runoff point with Flit-type handsprayers. This method gave complete disease control (Fig. 1) with either of the 4 oils.

Several types of inocula were used. Spore-free suspensions of mycelial fragments were prepared by placing vegetative colonies from 2 tubes of 10-day-old cultures in a sterilized Waring blender with 100 ml of sterile distilled water and macerating 1 minute. Inoculum containing conidia was prepared in 2 ways. Five ml of water with 0.1% Triton X-114[7] wetting agent were added to each of 3 tubes of a sporulating strain of *C. musae*, and agitated to free the cultured spores. Leaf material with Sigatoka spots was used to provide wild-type conidia for inoculum. After washed leaf-spots were incubated overnight in a mist chamber, the newly formed spores were removed with a camel's-hair brush and transferred to sterile distilled water.

RESULTS.—*Germination of conidia.*—The effect of several oils on the germination of conidia was studied. Three materials—pharmaceutical mineral oil, Silicone 555, and Orchard Spray Oil 35—were sprayed on young banana leaves in the field using 1 leaf per oil. Two leaf samples, 3×1 in., were removed from each treated leaf 24 hours later and inoculated in 6 areas with a water suspension of wild conidia. Leaf pieces sprayed with Bordeaux were inoculated at the same time as unsprayed pieces. When the drops of inoculum had dried, the leaf pieces were incubated 3 days in a mist chamber, removed, and examined for spore germination. At least 50 spores were counted in each of the 6 inoculated areas per treatment.

The data showed that germination of conidia was not stopped by oil sprays applied before inoculation.

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In cooperation with the United Fruit Company, Boston, Mass., and Esso Research and Engineering Company, Linden, N. J.

[2] Plant Pathologist, Assistant Officer in Charge, Research Assistant, and Agronomist, respectively.

[3] Information from United Fruit Co., 80 Federal St., Boston, Mass.

[4] Esso Research and Engineering Co., Linden, N. J.

[5] Heavy Californian liquid petrolatum sold by E. R. Squibb & Sons, N. Y.

[6] Dow Corning Corp., Midland, Mich.

[7] Rohm and Haas Co., Philadelphia, Pa.

The average germination of 2 tests for each of the treatments was: control, 53.7%; Silicone 555, 56.7%; Orchard Oil 35, 41.1%; pharmaceutical mineral oil, 39.7%; and Bordeaux 0.0%. The length and vigor of the germ tubes of all germinated spores were about equal.

Additional tests on the germination of conidia were conducted in the field to compare the effect of applying oil either before or after inoculation. The youngest leaves of 2 plants were marked with 3 8-in. bands across the upper surface. Several circles 1 in. in diameter were marked in each band. On 1 leaf the outer 2 bands were sprayed, one with pharmaceutical mineral oil and the other with Orchard Spray Oil 35. Twenty-four hours later, all circles on both leaves were inoculated with a water suspension of cultured conidia. Two hours later, the oils were applied, one to each of the outer 2 bands of the unsprayed leaf. After 10 days, the inoculated areas of each band were examined for spore germination. The results of 2 such tests showed that the germination of spores on oil-treated leaves was equal to that on the controls.

FIG. 1. An example of Sigatoka disease control by oil on a banana leaf. The left half received one application of pharmaceutical mineral oil; the right half was unsprayed.

Germination of ascospores.—Tests were conducted to determine the effect of oils on ascospore germination. Petri dishes containing water agar were prepared in duplicate, as follows: a) a layer of pharmaceutical mineral oil, or b) a layer of Orchard Spray Oil 35, and c) control. The depth of the oil was 0.5 cm. The dishes were covered with moistened pieces of diseased banana leaves. Ascospores discharged into the oil sank to the water agar, and at least 50 per plate were examined for germination at 24 hours. Two such tests showed more than 95% germination in all plates. The vigor of the germ tubes of spores in oil appeared equal to that in control plates. The test oils did not inhibit ascospore germination.

Stomatal penetration.—A study was made to determine the effect of oil on stomatal penetration by *M. musicola*. One half of the lower surface of the youngest leaf on 3 small Gros Michel banana plants was sprayed with pharmaceutical mineral oil; 24 hours later, both the treated and untreated halves were inoculated with a suspension of cultured conidia. The plants were kept in the open.

Eight days later, inoculated leaf sections were removed from both halves, stained with a crystal violet solution, and examined microscopically. Of 300 germ tubes observed on the treated leaf pieces, and an equal number on the controls, 21% and 29%, respectively, penetrated the leaf surface. The treatment significantly lowered penetration, but seemingly not enough to account for disease control.

Mycelial growth.—The effect of oil on mycelial growth was tested in culture, using Orchard Spray Oil 40, pharmaceutical mineral oil, and Silicone 555. Flasks, prepared in triplicate for each oil, contained 99 ml of liquid—either pure sterile oil or a 1:2 or 2:1 mixture of sterile oil and Phytone broth.[8] Controls consisted of 3 flasks containing 99 ml of Phytone broth. All flasks were inoculated with 1 ml of a macerated mycelial suspension, which was kept in intimate contact with the oil by maintaining the flasks on a rotary shaker for 8 days at room temperature. The resulting mycelial growths were compared visually. Growth was copious and equal in all flasks except those containing oil alone, where the absence of visible growth was probably due to the lack of water and nutrients.

A second test of the effect of oils on the vegetative growth of *M. musicola* compared 2 methods of prepar-

[8] Papaic digest of soya meal sold by Baltimore Biological Laboratory, Inc., Baltimore, Md.

TABLE 1.—*Conidial sporulation of M. musicola in the laboratory on banana leaf-spots 24 and 48 hours after spraying with petroleum oil, Silicone, or Bordeaux mixture*

Treatment	Index [a] of conidial sporulation [b]	
	At 24 hours	At 48 hours
Control	712	1559
Orchard Oil 40	770	1875
Mineral Oil	634	1448
Silicone 555	992	1315
Bordeaux	100	140

[a] Total rating of sporulation for all acervuli on 80 spots. Each acervulus is rated as follows: 0 = no spores; 0.5 = 1-9; 1 = 10-20; 2 = >20.

[b] No significant difference (at 5% level) between control and any oil treatment. Bordeaux is significantly lower (at 1% level) than all other treatments, as determined by Duncan's Multiple Range Test (4). Spore samples from each treatment showed at least 70% germination.

ing inoculum. A mycelial mat was macerated in pharmaceutical mineral oil, and the suspension allowed to stand 2 hours before use. A similar preparation was made in distilled water. Each mycelial suspension was used to inoculate a series of tubes containing various ratios of oil and Phytone broth. After 11 days of incubation, abundant growth resulted from both types of mycelial inocula. These data show that, in culture, the oil does not inhibit mycelial growth.

The possibility was checked that autoclaving had rendered the oils innocuous, but sterile oils sprayed on field plants gave complete disease control while untreated leaves had abundant disease.

Ascospore discharge.—The effect of oil on ascospore discharge was tested in 2 experiments with diseased leaves known to contain *M. musicola* ascospores. The spray treatments consisted of Orchard Spray Oil 35, pharmaceutical mineral oil, Silicone 555, and Bordeaux mixture. In each treatment, the distal half of 2 intact leaves in the field was sprayed, and the proximal half left untreated. At 4 and 6 days after spraying, samples were removed from the treated and untreated portions of each leaf. The samples were tested for ascospore discharge by wetting them, suspending them overnight in a saturated humidity over water-agar plates, and examining the agar surface for ascospores the following day.

Ascospores were discharged in approximately equal numbers from all the sprayed and unsprayed leaf samples. More than 90% of the ascospores in every plate had germinated and had robust germ tubes. Oil sprays, therefore, had no effect on inhibiting ascospore discharge at 4 or 6 days after spraying.

Production of conidia.—For the effect of petroleum and silicone oils on the production of conidia on banana leaves, Sigatoka spots were freshly cut from leaves, rinsed gently to remove any conidia already present, and after drying briefly, were treated and placed on moist filter paper in Petri dishes. The treatments were: a) Orchard Spray Oil 40, b) pharmaceutical mineral oil, c) Silicone 555, d) Bordeaux 5-5-50, and d) control. There were 80 spots in a total of 10 Petri dishes per treatment. The leaf spots were sprayed with a water mist 1 hour after treatment, and the Petri dishes were then covered. Spore counts on each spot and viability tests were made 24 and 48 hours later. The data are summarized in Table 1. It is apparent that the oil sprays did not inhibit the formation of conidia within 48 hours after spraying. Bordeaux, on the other hand, significantly reduced the production of conidia.

A field test was performed to determine whether oil sprays would develop an inhibition of sporulation over a 2-week period. Thirty spots on an intact leaf were marked and washed to remove all conidia. Ten randomized spots per treatment were circled, numbered with a crayon, and sprayed. The treatments were: a) Orchard Spray Oil 40, b) pharmaceutical mineral oil, and c) control. These treatments were replicated on 6 leaves of the same age on different plants. The spots were observed at 1, 3, 7, 12, and 14 days after the spray application. An increase in sporulation per spot from one observation date to another was considered new sporulation. In all treatments, abundant formation of new spores began before the third day and continued through the fourteenth day. The oils did not significantly develop an inhibition of conidial sporulation during the period studied.

DISCUSSION.—The chemical reactivity of the oils used here differs considerably. Commercial banana spray oils, such as Orchard Spray Oil Nos. 35 and 40, are relatively reactive, because they contain aromatic compounds, often comprising 10–25% of total volume. Pharmaceutical mineral oil, on the other hand, is one of the more stable petroleum oils available and is composed almost entirely of saturated hydrocarbons. Silicone Fluid 555, a synthetic oil, insoluble in water and soluble in certain organic solvents, is considerably more inert than the above petroleum oils and is very different from them in chemical structure. It is noteworthy that, although the above oils differ in chemical reactivity, all were found to control Sigatoka equally well. These results indicate that disease control can be obtained with oils differing widely in chemical composition and suggest that physical properties associated with oiliness may be important.

The oils tested did not inhibit *M. musicola* either in culture or on the leaf surface, even though the application rate was much higher than that used commercially. The oils therefore possibly inhibit the fungus inside the leaf, at some developmental stage after stomatal penetration and before symptom appearance. Studies are under way to determine the point where the fungus is stopped in the oil-sprayed leaf.

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UNITED STATES DEPARTMENT OF AGRICULTURE
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THE ASSOCIATION OF FUSARIUM WILT WITH THE ASPARAGUS DECLINE AND REPLANT PROBLEM IN CALIFORNIA[1]

R. G. Grogan and K. A. Kimble[2]

SUMMARY

A slow decline in the productivity of old asparagus plantings in California and an inability to reestablish productive plantings are due to damage of the root and vascular system by pathogenic organisms. The most prevalent and pathogenic organism associated with the disorder is *Fusarium oxysporum* f. *asparagi*, which is commonly seedborne as a surface contaminant.

Asparagus plantings in the Sacramento-San Joaquin Delta of California have an average profitable life that varies, but is usually 10–15 years. Plantings on new land usually reach peak production in about the eighth year, after which the size and number of spears gradually decrease to the point where the planting becomes unprofitable to maintain. This is what is meant by "decline."

Where plantings have declined, it is difficult, and usually impossible, to reestablish a commercially profitable stand. According to Hanna (4), replanting between old asparagus rows on Ryer Island immediately after plowing out the old stand has never produced a yield more than half of normal. Where asparagus has been direct-seeded on old land, emergence was reduced, and practically all seedlings yellowed, wilted, and died within 2–3 months.

California asparagus growers have circumvented this problem by shifting to land virgin to asparagus. However, a large part of the land in the Delta area is now, or has been, planted to asparagus, and a control is therefore needed to save the area from being forced out of production.

This paper reports on experiments to determine the cause of the problem. The evidence indicates that *Fusarium oxysporum* f. *asparagi* Cohen & Heald is a major factor.

Symptoms in declining or replanted stands.—Numerous observations, made to determine if any consistent symptoms were associated with poor growth, had the following results:

1) *Roots or rootlets.*—Small feeder rootlets arising from fleshy storage roots were rare. When present, they showed reddish discolored areas or were completely dead (Fig. 1C). A reddish streaking sometimes extended from a small discolored feeder root for 5 or more in. on the surface of the fleshy roots. The vascular system of the fleshy feeder roots often showed reddish-brown discoloration (Fig. 1C). Some of the fleshy roots had collapsed completely, leaving only the outer shell intact. Soft rot bacteria or other secondary organisms were usually associated with such collapse.

2) *Crowns.*—Reddish discoloration of the vascular tissue extended into the crown from discolored fleshy roots or from where a spear had been harvested (Fig. 1D).

3) *Ferns and stalks.*—Reddish-brown vascular discoloration in the fernstalks often extended several inches aboveground (Fig. 1A). Such ferns were often yellowed and stunted. Belowground, the bases of some stalks had rusty flecks and sunken lesions (Fig. 1B).

4) *Isolations and inoculations.*—The symptoms suggested that the decline might be due to a vascular wilt or root rot. Consequently, numerous isolations were made from discolored belowground parts; other isolations were made from seedlings growing in the greenhouse in soil taken from around plants showing symptoms. A variety of organisms were isolated in pure culture. Tests for pathogenicity of 443 of the isolates were made by inoculating asparagus seed at the time of planting—with spore suspensions of the organisms that produce spores, or with mycelial suspensions of the several non-sporulating isolates.

The most prevalent organism was *Fusarium oxysporum*. Of 317 isolates tested, 48% killed 75–100% of the inoculated seedlings, 20% killed 50–74%, 13% killed 25–49%, and 19% killed fewer than 25% or were non-pathogenic. Retests of a number of the

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[2] Associate Professor of Plant Pathology and Principal Laboratory Technician, respectively.

isolates indicated that the differences in pathogenicity were real and could be repeated. Eight isolates of *Fusarium solani* (Mart.) App. & Wr. emend. Snyd. & Hans. and 3 isolates of *Fusarium roseum* (Lk.) emend. Snyd. & Hans. were non-pathogenic. Of 21 isolates of *Fusarium moniliforme* (Sheld.) emend. Snyd. & Hans., 28% killed 75–100% of the seedlings, 33% killed 50–74%, 28% killed 25–49%, and 9% killed fewer than 25% or were non-pathogenic. Twenty isolates of *Phoma terrestris* Hansen all induced considerable root lesioning (reddish discolorations of roots and rootlets, somewhat similar to pink root of onion), but did not kill any of the seedlings. Of 13 isolates of *Rhizoctonia solani* Kuhn, only one was slightly pathogenic, causing slight lesioning near the crowns. Of the 61 other isolates—representing the genera *Ceratostomella*, *Sclerotinia*, *Botrytis*, *Zopfia*, *Chaetomium*, *Penicillium*, *Aspergillus*, and an unidentified non-sporulating grey culture—only one, a *Penicillium* species, was pathogenic, and that was slight. The most prevalent and virulent organism was *Fusarium oxysporum* f. *asparagi* Cohen & Heald (1). The pathogen is widespread, having been reported from several of the United States (1, 3). Ramsey and Wiant (5) reported it to be one of the most destructive diseases of asparagus shoots during transit.

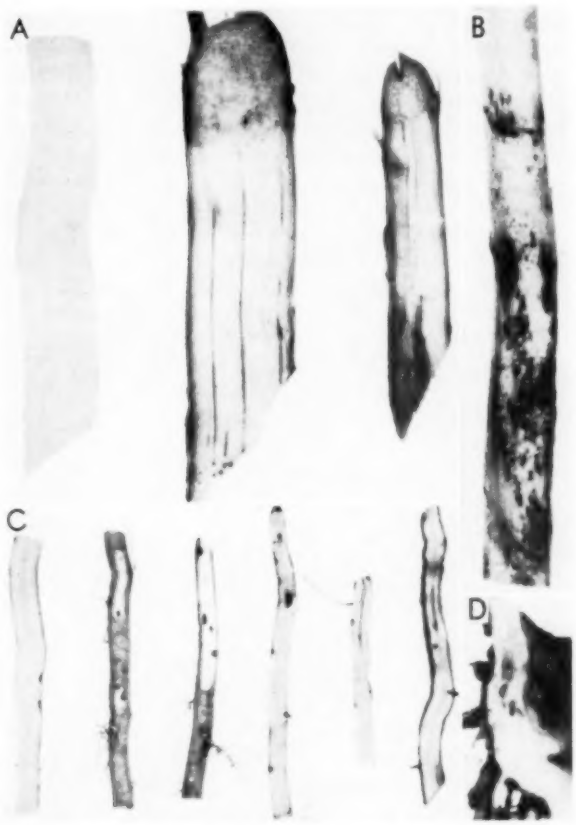

Fig. 1. Symptoms of Fusarium wilt of asparagus. **A)** Healthy portion of a stalk on the left; 2 stalks showing vascular discoloration on the right. **B)** Rusty colored flecks and sunken lesions on the belowground portion of a stalk. **C)** Healthy storage root on left, and several on right showing brown discoloration at points of attachment of lateral rootlets, and vascular discoloration in the 2 roots on the right. **D)** Vascular discoloration in a portion of an infected crown.

Reciprocal inoculations with specific forms of Fusarium oxysporum.—Cohen and Heald (1) reported that the *Fusarium* organism they had isolated from asparagus did not attack potatoes, tomatoes, or carnations, nor was it able to rot potato tubers or onion bulbs in moist chambers at 25°C. Only asparagus was vigorously attacked, although the fungus caused on Alaska peas a slight wilt that resembled the near wilt disease. To determine whether the *Fusarium* isolated from asparagus in California would attack other hosts, and whether the Fusarium wilt organisms specific for other hosts would attack asparagus, flax var. Dakota, watermelon var. Klondike, cotton var. Acala, tomato var. Pearson, sweet potato var. Puerto Rico, pea var. Alaska, bean var. Sutter Pink, and alfalfa var. California Common were inoculated by pouring a spore suspension of the asparagus Fusarium wilt organism over the seed and in the soil at the time of planting. Asparagus was similarly inoculated with Fusarium wilt organisms specific for the above-mentioned crops, obtained from stock cultures maintained at Davis, California. Symptoms resulted only in asparagus inoculated with the asparagus wilt organism.

Fusarium oxysporum, isolated readily from the rusty-brown flecking on the belowground portions of the asparagus spears, proved very pathogenic on seedlings. Harvested asparagus also was vulnerable. Spears washed 30 minutes in running water, placed in moist chambers, and sprayed with spore suspensions of one of the asparagus isolates showed lesioning and rotting within 7 days. No such damage developed in spears uninoculated or inoculated with *Fusarium oxysporum* specific to watermelon or to flax.

Inoculation of three-month-old crowns.—To determine the effect on older plants of inoculation with the asparagus Fusarium wilt organism, 45 3-month-old asparagus crowns were dipped in a spore suspension and transplanted; at the same time, 20 crowns were dipped in sterile water and transplanted. All crowns dipped in the spore suspension died within a month; all dipped in water survived and grew normally.

Movement of the Fusarium wilt organism in the soil.—Thirty asparagus seeds per row were planted in 1-in.-deep trenches in flats of steam-sterilized peat soil. A spore suspension of the asparagus Fusarium wilt organism was poured directly on the seed before covering, or placed 1 in. deep and 1, 2, 3, 4½, or 5 in. distant from the seed. With inoculum on the seed, an average of 2 plants emerged per row, and all died shortly after emergence. With inoculum 1 in. away from seed, an average of 26 plants emerged, of which only 2 wilted and died (in 27 days). Inoculum at greater distances showed no effect in 3 months. Results were similar when greenhouse compost soil was used. The Fusarium wilt organism apparently moves very slowly through the soil.

TABLE 1.—*Effect of different rates of seed treatment on emergence and survival of seedlings for 30 days in Fusarium-wilt-infested or uninfested soil*

Seed treatment (oz. of Lignisan per 100 lb. seed)	% emergence		% survival	
	Uninoculated soil	Inoculated soil	Uninoc. soil	Inoc. soil
0 (no treatment)	95	5.0	100	0.0
1	87	86.5	100	46.5
2	94	89.5	100	51.5
4	83	80.0	100	61.0
8	33	38.0	100	68.5

Seed artificially infested by wetting with a spore widespread distribution of asparagus Fusarium in plantings in the state, and its occurrence in plantings that have been started by direct seeding in soil new to asparagus suggested that the organism might be seed-borne. To test this possibility, 50-g samples of seed from 11 different lots were washed with 150 ml of sterile water, and the wash water from each sample was poured onto 50 surface-sterilized seeds planted in sterile soil. Three 50-seed samples were not inoculated, and averaged 47 emerging seedlings and no wilt symptoms. In all lots except one, wash water reduced surviving plants. The numbers of surviving plants were as follows: 46, 36, 30, 28, 26, 26, 23, 14, 13, 12, and 6. All wilted plants yielded the asparagus Fusarium wilt organism. In the lot with 46 plants surviving, no wilt was noted and no wilt organism found. The organism was apparently present on the surface of the seeds, but the method gave no information concerning the percentage of infested seeds.

Therefore, 6,000 unsterilized seeds were planted at 1-in. intervals in steam-sterilized soil. Of 5,640 that emerged, 5 wilted and died, and cultures from them yielded *Fusarium oxysporum* that was pathogenic on asparagus seedlings. In another test, two 5,000-seed samples from a commercial seed lot of the 873 variety were planted in steam-sterilized soil in flats in the greenhouse. One lot was first slurry treated with Lignisan (active ingredient 6¼% ethyl mercury phosphate) at the rate of 2 oz. per 100 lb. of seed. Emergence was 93.6% for the treated samples, and 92.8% for the untreated. None of the seedlings from the treated seed wilted, but 11 seedlings (about 0.22%) from the untreated seed wilted and died within 30 days of emergence. All wilted seedlings yielded *F. oxysporum*. In view of the success of the surface seed treatment, it seems probable that the seeds were infested on the surface only. The percentage was very small, but doubtless sufficient to be an important source of inoculum by which the organism can be introduced into uncontaminated areas.

Seed artificially infested by wetting with a spore suspension, and dried at room temperature, was slurry treated with Lignisan at the rate of 2 oz. per 100 lb. of seed and grown in steam-sterilized soil in the greenhouse. Comparisons were made with infested but untreated seed: 93% of the untreated seed germinated and produced apparently normal seedlings, but 41 of the 8,329 seedlings (0.5%) wilted and died within 30 days. The Lignisan seed treatment did not increase percentage of emergence, but no wilting, dying, or lesioning of roots was apparent in the 3,055 seedlings in this treatment.

The effect of seed treatment on emergence and survival of seedlings in Fusarium-infested or sterile soil was tested at different rates of Lignisan. The data are reported in Table 1. Reduced emergence indicated that 8 oz. per 100 lb. was phytotoxic, but all the other rates (1, 2, and 4 oz. per 100 lb.) increased rate of emergence, though none controlled wilting and dying after emergence.

Vertical distribution of the organism in field soil.—Samples of soil at 1-ft. intervals down to 5 ft. were brought into the greenhouse, and 25 seeds were planted in each of 4 6-in. pots of soil from each sampling depth. The asparagus Fusarium was present at all 5 depths, though least concentrated at 1 and 5 feet and most concentrated at 2 ft. (Table 2).

Fumigation and steam-sterilization of infested soil.—Fusarium-infested field soil was compared with portions of the same soil steam-sterilized or treated (1 ml per gal. of soil) with DD (dichloropropene dichloropropane), EDB (ethylene dibromide), chloropicrin, or CBP 55 (chloro bromo propene). The chemicals were injected into the soil in containers that were kept closed for 48 hours and then aerated for 6 days. Seeds were planted, and emergence and survival for 1 month were recorded. In untreated field soil, 68% of the seedlings wilted and died, and the survivors were stunted and showed considerable root damage. In contrast, no seedlings in steam-sterilized soil died or

TABLE 2.—*Inoculum concentration in infested field soil samples from different depths, as indicated by pre- and post-emergence killing of seedlings*

Soil depth (ft.)	No. seedlings emerged from 25 seeds	No. seedlings alive at 3 weeks
1	24	14
	19	10
	22	16
	22	14
2	9	0
	7	0
	13	4
	17	6
3	25	7
	12	1
	14	4
	25	1
4	24	3
	25	7
	20	2
	22	8
5	22	14
	24	7
	25	23
	24	19
Steam-sterilized soil (control)	23	23
	25	25
	25	25
	24	24

showed root damage. CBP 55 and chloropicrin were almost as effective as steam-sterilization in preventing root lesioning and seedling killing, but the pathogen was not completely eliminated: in the respective treatments, 1 and 5% of the seedlings wilted and died. Plants in EDB-treated soil grew fairly well, but roots showed considerable damage, and 7% of the plants wilted and died. DD was practically ineffective: growth was much stunted, root damage and wilting were severe, and 45% of the emerged seedlings died.

Mass testing to develop resistant varieties.—Any source of resistance to Fusarium wilt in horticultural types of asparagus adapted to California conditions would very likely be found among breeding lines at Davis that have been selected for longevity and have maintained production in old stands. Seeds of some of these lines were obtained from G. C. Hanna, plant breeder in the California Agricultural Experiment Station, and tested for resistance to Fusarium. Asparagus seed of the breeding line under test was planted thickly at a depth of $\frac{3}{4}$ in. in steam-sterilized soil that was inoculated with 2 virulent isolates of the asparagus Fusarium. Inoculations were made by thoroughly mixing in 0.2 g of ground-oat inoculum per cu. in. of soil. Greenhouse air temperatures were maintained at 25–28°C.

In a typical test the seedlings emerged very thickly but soon commenced dying. Very few were left at the end of 6–8 weeks, an average of about 1 plant in 5500 seeds (0.02%). The surviving plants were saved, and males and females were crossed to produce F_1 progeny, which, in turn, were tested as described above. In one such test, 168 plants from 3882 seeds survived and developed normally (4.3%). This percentage was low, but considerably greater than in the test of the parent material. Selection and testing of surviving progeny will be continued with the hope that a Fusarium-wilt-resistant strain can be developed.

DISCUSSION.—Although several pathogenic fungi are associated with the decline and replant problem of asparagus in California, the widespread occurrence and abundance of *Fusarium oxysporum* f. *asparagi* indicate that it is the most important pathogen involved in causing the disorder. This organism is highly pathogenic, as indicated by the rapid killing of seedlings in infested soil and the death of older crowns when dipped in spore suspensions prior to transplanting. One might expect a virulent organism of this type to cause rapid depletion and quick killing of infested plantings. However, its slowness of movement through soil suggests that considerable time would be required for the mass of soil around the root system to become completely infested. Also the mixing of infested with uninfested soil when a planting is removed might explain why root damage and seedling killing are more severe on replanted land than was evident in the previous planting.

The Fusarium wilt of asparagus in California is similar to, and probably identical with, the disease described by Cohen and Heald (1), who, in fact, reported the isolation of the causal organism from infected asparagus plants received from California. Although it is possible that the Fusarium-induced seedling disease of asparagus reported by Graham (2) is caused by the same organism, his report indicates that he was dealing with a less virulent group of clones, a group incapable of vascular invasion. The clones studied herein caused severe damage to cortical tissues, and were also capable of invasion, movement in, and discoloration of the xylem, which is typical of the vascular Fusaria. Also, isolates obtained from discolored xylem elements several inches aboveground were highly virulent, indicating that the organism is a vascular parasite.

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CHENOPODIUM HYBRIDUM AS A LOCAL-LESION ASSAY HOST FOR BROME MOSAIC VIRUS[1]

W. F. Rochow[2]

SUMMARY

Brome mosaic virus consistently induced distinct necrotic local lesions on leaves of *Chenopodium hybridum*. When Carborundum-dusted leaves were inoculated, about 20 times as many lesions developed as when no abrasive was used. Brushing inoculum on leaves produced more lesions than other rubbing methods. More lesions resulted from neutral 0.1*M* phosphate buffer as diluent than from other diluents tested, but partly purified virus preparations stored at 3°C in neutral phosphate buffers were less infectious than similar preparations stored in distilled water or in 0.1*M* acetate buffer, pH 5.1. Lesion numbers were essentially inversely proportional to dilution of inocula. Inocula differing in virus content by 20% or more showed statistically significant differences in lesion numbers when assayed on 36 opposite half-leaves. A 4-solution inoculation scheme based on 72 leaves on 12 plants was satisfactory for estimating differences in relative virus activities of 2 inocula by the 2-dilution potency assay method. These data show that plants of *C. hybridum* are reliable for local-lesion assay of brome mosaic virus.

Several local-lesion hosts have been reported for BMV (brome mosaic virus, *Marmor graminis* McK.) (3, 4), but none proved satisfactory for routine quantitative infectivity assays in this laboratory. Some additional hosts were therefore tested. No advantage over those previously reported was found when plants of *Gomphrena globosa* L., *Vigna sinensis* (L.) Savi, *Chenopodium urbicum* L., and *C. quinoa* Willd. were inoculated with BMV, although chlorotic or necrotic local lesions usually formed. Inoculated plants of *Chenopodium hybridum* L., however, consistently developed clear necrotic local lesions in tests made over a period of 2 years. This species has also been reported as a local-lesion host for cucumber mosaic virus (7) and eggplant mosaic virus (1). This paper reports the results of tests of *C. hybridum* as a local-lesion host for the quantitative estimation of BMV activity.

MATERIALS AND METHODS.—Inoculations were made in a greenhouse maintained at about 70°F except during the summer, when temperatures sometimes rose to 90° or higher. BMV was supplied by H. H. McKinney as isolate No. 66 of the American Type Culture Collection. The virus was maintained by serial transfer, usually in Michigan Amber wheat (*Triticum aestivum* L.). Crude juice inoculum, used except where stated otherwise, was prepared from plants that had been infected about 2 weeks. Juice was obtained with mortar and pestle and strained through 2 layers of fine cheesecloth.

For some inoculations, preparations of BMV were partially purified: Crude juice was clarified by freezing and by low-speed centrifugation. Preparations were carried through 3 cycles of differential centrifugation. Low-speed centrifugation was done with a Servall model SP centrifuge; high-speed centrifugation was done at 144,000 G in a refrigerated Spinco model L centrifuge. Final virus pellets appeared homogeneous and light brown. The content of virus material in these preparations was estimated by a micro-Kjeldahl method.

Seeds of *C. hybridum* were originally supplied by D. A. Roberts. Poor germination of seeds, usually below 1%, has been a major problem in using this host; a procedure usually giving about 10% germination, sufficient for routine production of plants, was developed. The treatment consists of immersing seeds 3–5 minutes in concentrated H_2SO_4 and leaching about 1 week under a dripping water tap.

Treated seeds were planted in vermiculite, and the seedlings were transplanted to steam-sterilized soil in 4-in. pots. Supplemental illumination was provided to extend day length until at least 6 fully expanded leaves had formed. Plants were ready for use 5–7 weeks after the seeds were sown. All leaves except those to be inoculated were removed at least 1 day before inoculation. After inoculation, all leaves were rinsed with tap water, although rinsing did not appear to affect lesion formation. Distinct, necrotic local lesions were usually first visible after 3–5 days, and well formed after 10–15 days, when they were counted (Fig. 1).

Since leaves of *C. hybridum* are large and symmetrical, all inoculation procedures were based on the use of opposite half-leaves. The relative infectivities of single dilutions of each of 2 inocula were compared directly by inoculation of opposite half-leaves in a balanced pattern. For a comparison of the relative infectivities of 4 inocula, a balanced 4-solution inoculation scheme was developed for use on 12 plants, each with 6 leaves (Table 1). This scheme was also used for estimating how greatly 2 inocula differed in infectivity, by the potency assay method of Spencer and Price (11).

Phosphate buffers were prepared by mixing equimolar solutions of $Na_2HPO_4 \cdot 7H_2O$ and KH_2PO_4 to obtain a pH of 7.0, determined with a glass electrode. Acetate buffer was prepared by mixing 0.1*M* acetic acid with 0.1*M* sodium acetate to produce a pH of 5.1.

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USE OF CARBORUNDUM.—In a test of the effect of 400-mesh Carborundum on the number of lesions formed, an inoculum prepared by dilution in 0.1*M* phosphate buffer was divided into 2 parts. One part was brushed on alternate right and left half-leaves of 6 leaves on each of 4 plants. After the plants were dusted with Carborundum, the opposite half-leaves were brushed with the other part of the inoculum. Only 11 lesions formed on the undusted half-leaves, whereas 220 lesions formed on the dusted half-leaves. In a second test the corresponding lesion totals were 9 and 188. In all succeeding tests, plants were dusted with 400-mesh Carborundum immediately before inoculation.

METHOD OF INOCULATION.—The 4-solution scheme (Table 1) was used to compare different methods of inoculation. Four identical inocula were prepared by diluting a sample of crude juice. Each inoculum was applied to 36 half-leaves—two inocula with a brush, one with a cheesecloth pad, and one with the forefinger. Each half-leaf was given 6 strokes in inoculation, except that one of the brush applications was at the rate of 12 strokes. Inoculum was renewed on brush or pad after each plant (3 half-leaves), and on the finger after each half-leaf. Lesion numbers were as follows: brush (6 strokes), 719; brush (12 strokes), 998; pad, 94; and finger, 304. In all other inoculations, brushes (Wilton No. 8 or No. 10) were used as described by Takahashi (12).

TABLE 1.—*Four-solution inoculation scheme used for assay of brome mosaic virus infectivity on plants of Chenopodium hybridum* [a]

Leaf position	Inocula used on indicated plants [b]											
	1		2		3		4		5		6	
6	A	B	C	D	B	C	A	D	C	A	D	B
5	C	D	A	B	D	A	C	B	D	B	C	A
4	B	C	D	A	A	C	D	B	B	A	D	C
3	D	A	B	C	B	D	C	A	D	C	B	A
2	A	C	B	D	A	B	D	C	C	B	A	D
1	B	D	A	C	C	D	B	A	A	D	C	B

[a] Capital letters refer to inocula brushed on; right or left half of leaf is indicated by relative position of letters. The lowest position for each solution was inoculated first on each plant, and inoculum was renewed on the brush after inoculation of each plant.

[b] The design for plant numbers 7–12 was the reverse of that shown; thus, the plan for plant 7 was the mirror image of that for plant 1, etc. The order of inoculation for plants 1–3 was A, B, C, D; for plants 4–6, D, C, B, A; for plants 7–9, C, A, D, B; for plants 10–12, B, D, A, C. Rinsing was done immediately after the inoculation of each of these 4 groups.

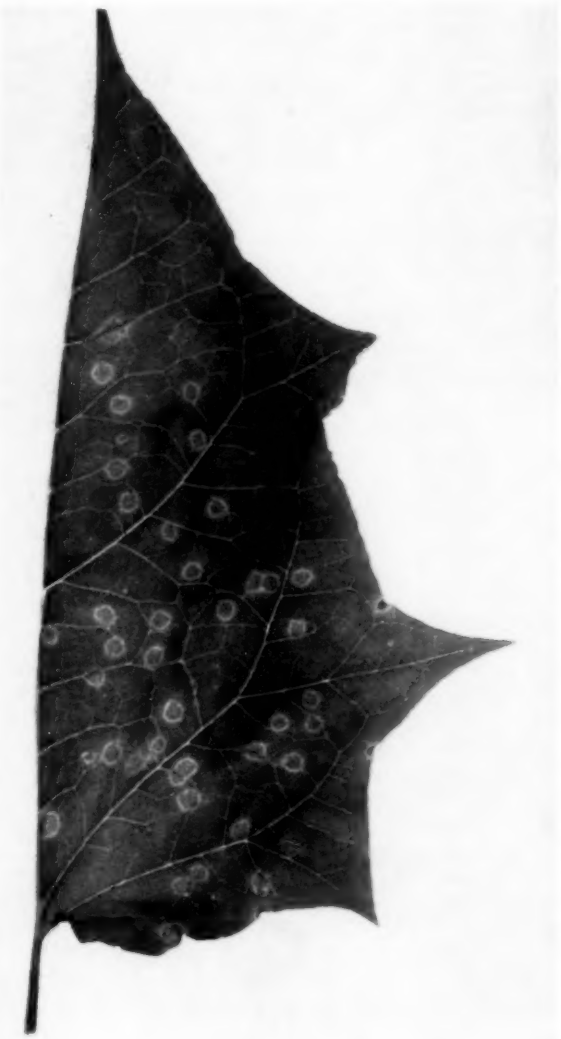

Fig. 1. Local lesions on half of a symmetrical leaf of *Chenopodium hybridum* 10 days after inoculation with brome mosaic virus. ×1. (Photograph by H. H. Lyon.)

Additional study was made of the importance to lesion formation of the amount of brushing. In tests involving the 4-solution scheme, the upper surface of each half-leaf was brushed so that its surface was covered 1, 2, 3, or 4 times. In 2 of 3 such tests, half-leaves brushed 3 times formed the most lesions, but the differences among treatments were small. The greatest difference was in a test in which brushing once gave 68 lesions, and brushing 4 times gave 194 lesions. Each half-leaf in all other tests was brushed so that its surface was covered twice.

DILUENT.—After preliminary tests on separate plants, the 4-solution scheme was used in studying the effect of different diluents on lesion formation. Four comparable inocula were prepared by equal dilution in 1) 0.1*M* phosphate buffer, pH 7; 2) 0.01*M* phosphate buffer, pH 7; 3) 0.1*M* acetate buffer, pH 5.1; and 4) distilled water. When crude juice was used as inoculum, the average number of lesions on 36 half-leaves for each of the 4 diluents was 64, 4, 4, and 23, respectively. A partly purified preparation of BMV produced corresponding averages of 41, 1, 8, and 11. Since the concentration of the phosphate buffer appeared to have a great influence on lesion formation, additional tests were made with phosphate buffers. The 4-solution scheme was used to compare neutral phosphate buffers at concentrations of 1.0*M*, 0.5*M*, 0.1*M*, and 0.01*M*. In 1 test the average numbers of

lesions on 36 half-leaves were 1, 1, 10, and 1, respectively. Lesion counts were even lower in 2 similar tests, but, again, the 0.1*M* concentration produced the most lesions. Except where stated otherwise, the diluent used in other experiments was neutral 0.1*M* phosphate buffer.

Although 0.1*M* phosphate buffer was the best diluent for assay, phosphate buffers did not appear to be the best solvents for storing partially purified preparations at 3°C. This effect was noted first, and most often, in comparing distilled water with 0.01*M* phosphate buffer. In the first purification of BMV, the initial juice was divided into 2 lots; during subsequent purification, distilled water was the suspending medium for 1 lot, and 0.01*M* phosphate for the other. The infectivities of the final preparations, containing equal quantities of virus material, were compared after 5 days. The diluted phosphate preparation failed to produce lesions on Golden Giant corn or on any of 14 inoculated half-leaves of *C. hybridum*, whereas a comparable dilution of the water preparation produced many lesions on corn, and 472 lesions on the 14 opposite half-leaves of *C. hybridum*. Marked differences in the infectivities of purified BMV stored at 3° were also noted several times in tests in which water was the suspending medium during purification and comparable final pellets were suspended either in distilled water or in 0.01*M* phosphate. In 1 such test, the 2 preparations, after 16 days' storage, were compared on opposite half-leaves of *C. hybridum* after dilution in 0.1*M* phosphate buffer to a final concentration of 0.27 mg of virus per ml for the phosphate preparation and 0.24 mg/ml for the water preparation. Lesion totals were 207 for the water preparation and 11 for the phosphate preparation.

TABLE 2.—*Sensitivity of opposite half-leaf assays on Chenopodium hybridum in detecting differences between 2 brome mosaic virus inocula diluted in neutral 0.1M phosphate buffer*

Virus content of "unknown" as % of "standard"	No. of lesions per half-leaf[a]	
	"Standard"	"Unknown"
100	35	36
100	40	42
100	42	39
90	52	46
85	30	23**
80	39	29**
80	39	25**
80	34	22**
80	31	24**
60	34	20**
40	29	9**

[a] Each figure is the average for 36 half-leaves. ** indicates that the difference between the "standard" and "unknown" was significant at the 1% level.

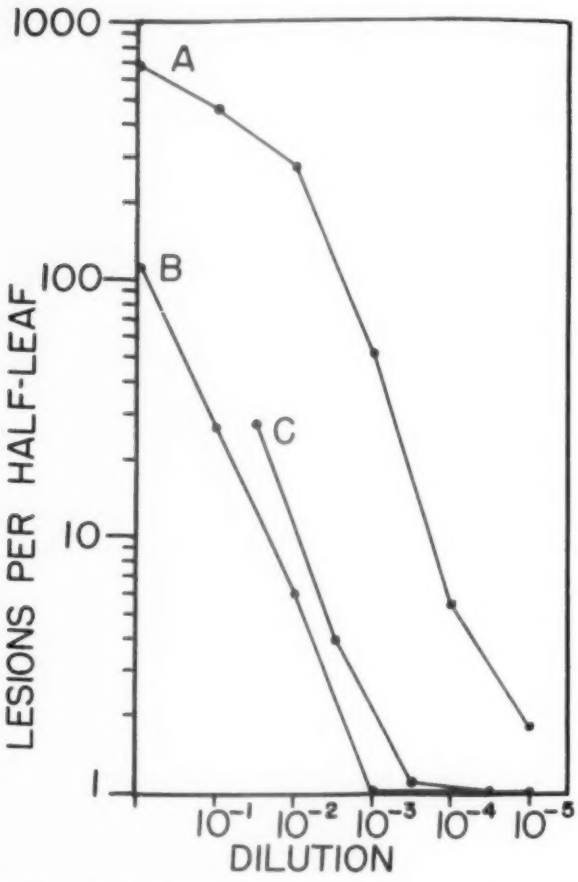

Fig. 2. Numbers of lesions obtained on plants of *Chenopodium hybridum* with different dilutions of 3 preparations of brome mosaic virus. Crude juice from infected Michigan Amber wheat was diluted either in neutral 0.1*M* phosphate buffer (Curve A) or in distilled water (Curve B). Partially purified virus (24 mg/ml in distilled water) was diluted in neutral 0.1*M* phosphate buffer (Curve C).

In one test the effects of storage of partly purified BMV at 3°C were compared in 1) 0.1*M* phosphate buffer, 2) 0.01*M* phosphate buffer, 3) 0.1*M* acetate buffer, and 4) distilled water. The relative infectivities of the preparations were compared by dilution in 0.1*M* phosphate buffer at intervals after comparable amounts of virus (20 mg/ml) were placed in each of the 4 solvents. After 7 days, for instance, the average number of lesions produced on 36 half-leaves by each of the respective preparations was 67, 9, 158, and 122.

DILUTION CURVE.—A 6-solution inoculation scheme similar to the 4-solution scheme (Table 1) was used to determine dilution curves of BMV in juice from infected wheat plants. The 4-solution scheme was used in a similar study of BMV diluted from a partly purified preparation. Lesion numbers were essentially inversely proportional to dilution (Fig. 2). These data suggested that *C. hybridum* should be useful for the quantitative estimation of BMV infectivity.

DETECTION OF DIFFERENCES IN INFECTIVITY OF 2 INOCULA.—Two inocula were prepared from a single sample of crude juice so that one could be considered a "standard" and the other an "unknown." The "unknown" was diluted so that it was either equal to the "standard" in content of BMV or differed by a known

TABLE 3.—*Accuracy of 2-dilution potency assays of brome mosaic virus on plants of Chenopodium hybridum*

Potency		No. of lesions per half-leaf by lower dilution of inocula indicated [b]	
Actual [a]	Measured	"Standard"	"Unknown"
0.20	0.23	57	9
0.30	0.24	6	1
0.50	0.43	9	3
0.80	0.73	4	3
1.00	0.96	65	73
1.00	1.21	21	18
1.00	1.26	5	7
2.00	1.91	6	13
3.00	3.25	20	50
4.00	2.37	12	22
5.00	5.74	6	19

[a] Actual ratio of virus content of "unknown" to that of "standard" based on dilution in neutral 0.1*M* phosphate buffer.

[b] Each figure is the average for 36 half-leaves of 12 plants.

amount. The relative infectivities of the 2 inocula were then compared on 36 leaves (6 plants) to determine the sensitivity of *C. hybridum* for detection of differences between the 2 inocula. Lesion counts were analyzed by Student's t-test (10). Two inocula differing 20% or more in virus content consistently gave differences that were statistically significant if lesion counts of the "standard" were in the range of 20–55 lesions per half-leaf (Table 2). In other tests in which lesion counts were outside this range, inocula differing by 20% were not distinguished consistently.

TWO-DILUTION POTENCY ASSAY.—The 4-solution scheme (Table 1) was used to investigate the possibility of using plants of *C. hybridum* to estimate relative differences in the infectivities of 2 inocula by the potency assay method of Spencer and Price (11) as used previously in this laboratory (8). In this procedure, a "standard" and an "unknown," each at 2 dilutions, are compared. An estimate of the accuracy of this system was obtained by assays of "unknowns" of known potency. Four dilutions of juice from BMV-infected wheat plants were prepared so that 2 dilutions could be considered the "standard" and 2 the "unknown." The 2 dilutions of each series differed by the same log interval, 0.5. The ratio of the infectivity of BMV in the "unknown" to that in the "standard," based on actual dilutions, was compared with the measured ratio (Table 3). Differences between actual potencies and measured potencies were not significant when analyzed by the t-test (10). Of the 11 comparisons (Table 3), 5 gave measured potencies higher than actual potencies, and 6 gave lower values. Differences were no greater than 20% except in 3 instances, in which measured potencies differed from actual potencies by 21, 26, and 41%. These data show that the error of this system is usually within the range of error of similar plant virus assay systems (5, 6, 8, 9, 11).

DISCUSSION.—Plants of *C. hybridum* meet the requirements outlined by Hollings (2) for an ideal test plant for virus work: the plant should be easy to raise; should remain susceptible for a long time; should have even, flat leaves large enough for use of the half-leaf method; and should produce discrete local lesions in numbers sufficient to minimize errors.

Plants of *C. hybridum* are generally easy to raise, but seed germination is poor. In the writer's experience, however, this disadvantage can be offset by preplanting treatment of seeds and by the fact that the large quantities of seed readily produced by plants of *C. hybridum* facilitate use of excess seed. A further possible disadvantage of the plant is its susceptibility to mite injury. Occasional fumigation injury has resulted from the use of smoke-generators for greenhouse fumigation.

C. hybridum remains susceptible to BMV for a long time, but susceptibility decreases as plants approach senescence. Leaves can be used for several weeks after they expand; young unexpanded leaves also produce local lesions. More lesions usually occur on lower leaves than on upper leaves. Uninoculated leaves of infected plants have remained symptomless, and BMV has not been recovered from them.

Since leaves of *C. hybridum* are large, flat, and symmetrical, they fulfill the requirements of the half-leaf method. Although plants usually were trimmed to 6 leaves in the tests reported here, more leaves can be used per plant. Leaves appear to be relatively delicate, but inoculation injury has been a problem on only a few occasions, when plants were wilted at the time of inoculation.

After inoculation with BMV, discrete local lesions form in sufficient numbers to minimize errors. It seems likely that the use of Carborundum is a major source of error in this assay system, since the abrasive has such a large effect on lesion formation. Although the amount of Carborundum does affect the number of lesions formed, the accuracy of an assay on dusted plants was equal to that of a comparable assay in which the Carborundum was suspended in the inocula.

Plants of *C. hybridum* may be less useful for diagnostic work than for quantitative work since many viruses cause local lesions on this host. Lesions caused by cucumber mosaic virus (7) particularly resemble those of BMV.

Although there are some disadvantages in the use of plants of *C. hybridum*, the author's experience indicates that this host should enable better estimation of BMV activity than do previously reported hosts.

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A SEEDLING REACTION OF WHEAT INDICATIVE OF BUNT INFECTION [1]

E. L. Kendrick and Laurence H. Purdy [2]

SUMMARY

When infected by bunt fungi, Red Bobs, a spring wheat susceptible to all known bunt races, exhibited a distinct and characteristic chlorotic flecking of leaves, from the 3- or 4-leaf stage to maturity. Partially infected plants were detectable by a flecking on only one side of the leaf mid-rib. These seedling reactions have considerable potential value in testing for bunt resistance.

Symptoms of bunt infection (*Tilletia* spp.) in wheat seedlings were observed and described as early as 1934. Thus far such early symptoms have not been recognized as infallible criteria for determining the susceptibility of wheat varieties. Churchward (1) reported white, opaque spots on young leaves of wheat plants inoculated with *Tilletia caries* (DC.) Tul., but noted that the spots occurred on both susceptible and resistant varieties. Johnston and Lefebvre (3) observed a chlorotic mottling of wheat leaves inoculated with *Tilletia foetida* (Wallr.) Liro and grown in the greenhouse. This same type of leaf mottling was also observed under field conditions, but was not easily differentiated from certain chlorotic disturbances attributable to malnutrition, diseases other than bunt, insects, and winter injury. Nevertheless, Johnston and Lefebvre predicted the susceptibility to bunt of many hybrid lines of winter wheat with fair success on the basis of leaf mottling of plants in the rosette stage of growth. Their classifications were most accurate for highly susceptible and highly resistant hybrid families. They decided that leaf symptoms alone should not be considered a conclusive measure of the amount of infection. Fischer and Holton (2) observed a distinct chlorotic striping and mottling of certain wheat varieties as a result of infection with dwarf bunt, *Tilletia contraversa* Kuehn.

Over a 3-year period, the spring wheat variety Red Bobs has been inoculated with *T. caries* and *T. foetida* in greenhouse experiments dealing with various phases of the bunt problem at Pullman, Washington. These experiments have shown a seedling reaction, expressed as a mottling or a flecking of the leaves, that may eliminate the necessity of growing this variety to maturity to determine whether the plant is infected.

MATERIALS AND METHODS.—Seed of Red Bobs, a spring wheat susceptible to all known races of bunt, was inoculated with races 1, 3, 5, 6, 9, 10, 12, 13, 14, 15, 16, 17, and 18 of *Tilletia caries* and races 4, 7, 8, 9, and 10 of *T. foetida*. All the pathogenic types of importance from a plant breeding standpoint are represented among these races. In some cases the inoculated seed was planted in sand and incubated at 10°C. In others the seed was planted in Palouse silt loam maintained at various moisture levels at temperatures of 5°, 10°, 15°, and 20°, and transplanted into greenhouse benches after various periods. Before transplantation, all seedlings were washed free of sand or soil and soaked 2 minutes in a 1:1000 water dilution of Panogen 15 (2.2% cyano[methylmercuri]guanidine) to prevent further infections by spores carried on the seed. The plants in the greenhouse benches were grown under controlled light and temperature conditions.

Four incandescent lamps over each bench supplemented natural daylight from 8 A.M. to 5 P.M., and the dark period was broken from 11 P.M. to 2 A.M. The air temperature was maintained at about 25°C during the day and 15° at night. Under these conditions each plant produced a single head. Thus, seedling counts are the same as head counts, upon which percentage of infection is usually based. Seedling reactions were observed and recorded in the 3- or 4-leaf stage, and seedlings showing flecking were tagged. At maturity, infected and uninfected plants were compared with tagged and untagged plants.

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[2] Pathologists, Crops Research Division, ARS, U. S. D. A., Pullman.

RESULTS.—A distinct and characteristic chlorotic flecking (Fig. 1-A,B) was exhibited by the leaves of Red Bobs wheat plants infected by the bunt fungi. This flecking was easily detectable from the 3- or 4-leaf stage up to maturity, when the flecking of the lower leaves was more pronounced, and occasionally

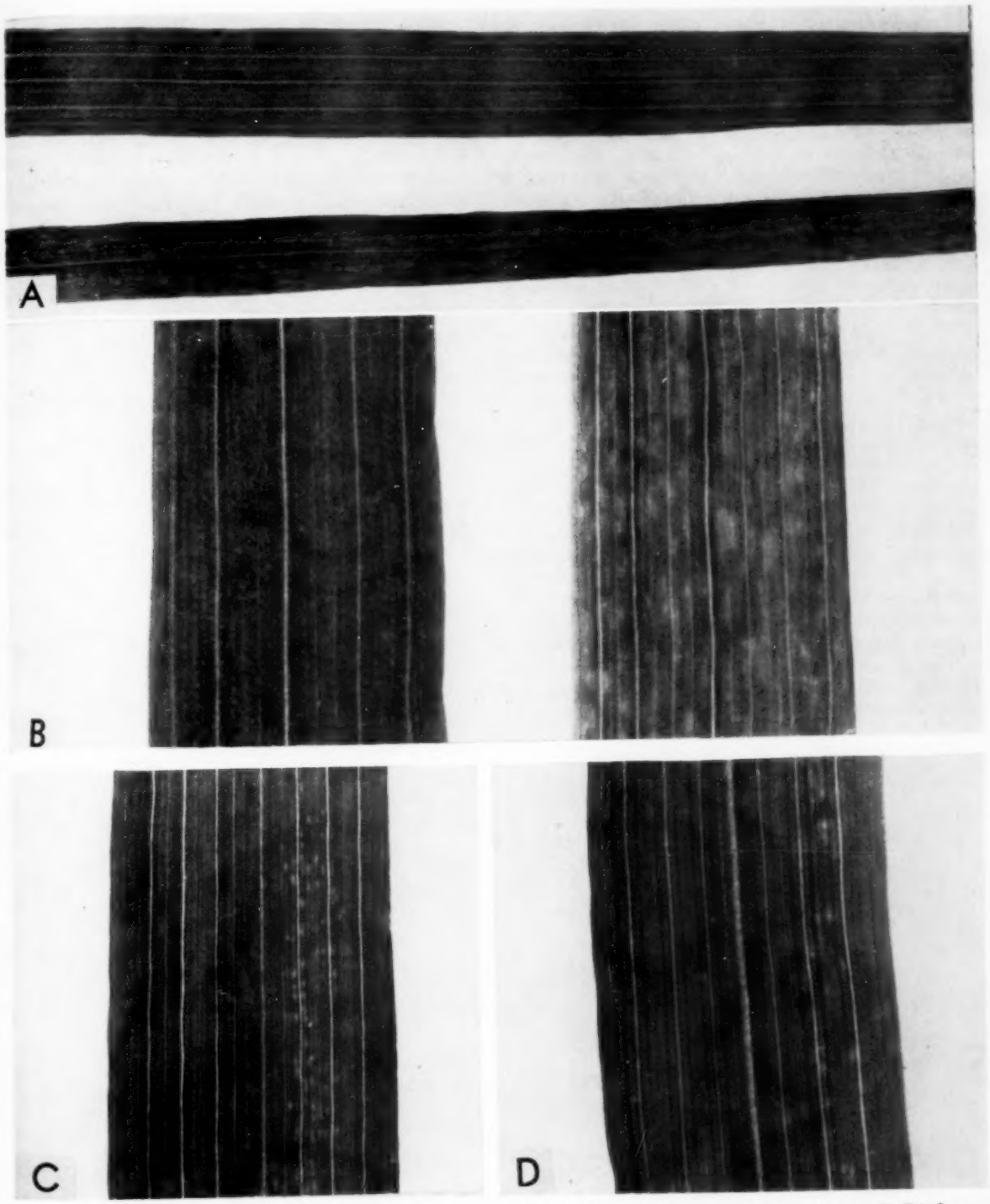

Fig. 1. Leaf flecking of Red Bobs wheat indicative of bunt infection (*Tilletia* spp.). **A)** Upper leaf from healthy plant; lower leaf from bunt-infected plant (×1). **B)** Portion of leaf from healthy plant (left) and from infected plant (right) at higher magnification (×3). **C, D)** Leaves from partially infected plants, showing unilateral flecking (×3).

resulted in a streaking of the basal leaves. This was generally associated with necrosis of the leaf tips and margins. The association between leaf flecking and bunt infection was invariable in experiments involving over 10,000 plants of Red Bobs in a 3-year period.

Partial infection of wheat heads was likewise associated with partial flecking of the leaf surface. Partially infected heads were frequently encountered in which there were bunt balls on one side of the rachis, or only a few in the entire head. Such heads could be predicted on the basis of flecking that characteristically appeared on only one side of the leaf mid-rib (Fig. 1-C,D). The density of such flecking usually indicated the type of partially infected head that would develop. A pronounced unilateral flecking (Fig. 1-C) generally forecast a partially infected head with bunt balls all along one side of the rachis, whereas mild or sparse unilateral flecking (Fig. 1-D) generally forecast a head with only a few bunt balls.

Discussion.—The seedling reaction described may become a valuable tool in other studies on bunt, such as those on the establishment and development of the bunt fungi in the wheat plant (4, 5). In such studies a susceptible spring wheat, such as Red Bobs, can be grown in the greenhouse throughout the year. Determining percentage of infection in the seedling stage eliminates the necessity of growing plants to maturity, saving as much time as 2 months.

Preliminary studies have shown that susceptible winter wheats exhibit the characteristic seedling flecking. Thus, this seedling reaction has considerable potential value in testing for bunt resistance in winter wheats. With this method it would be possible to screen a large number of winter wheat varieties in a relatively short time, for there would be no need for the winter vernalization period and subsequent growth to maturity. In the study reported here, certain varieties known to be highly resistant to all bunt races failed to show any flecking in the seedling stage, whereas all susceptible varieties exhibited the flecking reaction. Thus, there is a good possibility of using this seedling reaction in screening winter wheat varieties for resistance and susceptibility. However, as Churchward (1) and Johnston and Lefebvre (3) observed, some varieties known to be resistant also exhibit various degrees of flecking. There were some indications in this study that type of seedling reaction may be correlated with known types of resistance in winter wheats, but this relationship was not conclusive. Further study is needed of this and other aspects, such as influence of different bunt races and different sources of resistance in applying the seedling reaction to winter wheats.

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MANCHA GRIS, A NEW LEAF DISEASE OF BEAN IN COLOMBIA [1]

R. L. Skiles [2] and Canuto Cardona-Alvarez [3]

SUMMARY

About 1953 a new leaf disease of field bean was observed in Colombia. The causal fungus, *Cercospora vanderysti*, was reported previously only on *Vigna* spp. The symptoms and morphology of *C. vanderysti* distinguish it easily from *C. canescens*, which is commonly found in the Cauca Valley. Other *Cercospora* spp. that occur on bean were not seen in Colombia. *C. vanderysti* could not be cultured on any medium. The fungus is widely distributed at altitudes of 1500–2200 m and is a potentially serious disease. Some sources of resistance were found in foreign bean collections.

From 1953 to 1956, a leaf disease not recorded previously in Colombia was observed on field beans (*Phaseolus vulgaris* L.). The symptoms, first believed to be incipient ones of angular leaf spot (*Isariopsis griseola* Sacc.), were later determined to be produced by a distinct pathogen. The disease was named "mancha gris" (gray blotch), from its symptoms, and its causal agent was identified as *Cercospora vanderysti* P. Henn.[4]

SPECIES OF CERCOSPORA REPORTED ON PHASEOLUS AND RELATED HOSTS.—*C. vanderysti* P. Henn., according to Chupp (4), is synonymous with *C. vignae-vexillatae* Baker & Dale, and has been reported from Congo and Trinidad. The reported hosts, however, were not *Phaseolus* but *Vigna venulosa* Baker, *V. repens* Baker, and *V. capensis* Walp. (= *V. vexillata* (L.) Rich.). Some species of *Cercospora*, namely *C. canescens* Ell. & G. Martin, *C. cruenta* Sacc., and *C. dolichi* Ell. & Everh., are known to infect both genera; the similar host range of *C. vanderysti* is thus not unusual. In Colombia, where different introduced and local varieties and breeding lines of bean have often been grown adjacent to *V. sinensis* (L.) Endl. collections, no *Cercospora* species has ever been isolated from *Vigna*. The collections of the latter genus are, in fact, remarkably healthy in Colombia with respect to foliage diseases.

Zaumeyer and Thomas (5) and Chupp (3, 4) list the following species of *Cercospora* as reported on *Phaseolus* spp.: *C. canescens*, *C. cruenta*, *C. lussoniensis* Sacc., *C. caracallae* (Speg.) Chupp, *C. phaseolorum* Cke., *C. phaseoli* Dearn. & Barth., and *C. faseolina* Speg. (=*C. phaseolina* Speg.). In 1953, Chupp (4) reduced this number to 3: *S. canescens*, *C. cruenta*, and *C. caracallae*, with the others as synonyms or belonging to other genera.

SPECIES OF CERCOSPORA ON PHASEOLUS VULGARIS IN COLOMBIA.—The only species of *Cercospora*, aside from *C. vanderysti*, that the authors have found on bean in Colombia is *C. canescens*. The symptoms and other characteristics, such as the long (175μ), acicular, commonly and distinctly 9-septate conidia, were clearly typical of the species. In 1930, Chupp (3) described *C. faseolina* on *P. vulgaris* from the Cordillera Occidental (western mountain range) of the Cauca Valley, Colombia, in addition to *C. canescens* on a *Phaseolus* sp. from the Cauca Valley proper. The latter species is commonly found in the extensive bean fields of the level, arable lands of the Cauca Valley. The authors have surveyed bean diseases at higher altitudes to the south (though not in the mountains west of the Cauca Valley) and have not found *C. faseolina*. The distribution of the 2 species on *P. vulgaris* may be influenced by the regional distribution of the cultivated and wild varieties of the host and by the climatic extremes between the Cauca Valley and the surrounding mountains.

SYMPTOMS.—The early confusion between mancha gris and angular leaf spot arose because the leaf lesions caused by *C. vanderysti* are typically angular in outline, limited by the veins, and the same size as those caused by *Isariopsis griseola*. Cardona-Alvarez and Walker (2) and Barros et al (1) described the symptoms of angular leaf spot in detail.

The first symptom of *C. vanderysti* infection is a slightly chlorotic, angular lesion that is visible on the

FIG. 1. The upper surface of bean leaflets, showing the pale, angular lesions caused by *Cercospora vanderysti*. Many lesions are limited by the veins, and others have begun to coalesce. The small, dark, angular lesions are caused by *Isariopsis griseola*.

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[4] The authors are indebted to Drs. J. A. Stevenson, U. S. D. A., M. P. Backus, University of Wisconsin, and S. J. Hughes, Department of Agriculture, Ottawa, Ontario, for invaluable aid in taxonomic classification of the fungus.

upper surface of the leaf (Fig. 1). The spots are about 2–5 mm in diameter, and often so numerous that the leaf looks infected by the yellow bean mosaic virus. The individual lesions may later coalesce, covering areas up to 10–20 mm in diameter. Heavy infection causes premature defoliation.

Within the angular lesions the first sign on the upper leaf surface is a very fine, grayish-white, powdery growth of conidiophores and conidia, so slight as often to be invisible to the unaided eye; on preserved specimens it is rarely noticed. The most distinguishing sign of the disease is seen on the reverse side of the lesions: a gray, mat- or cushion-like fungus growth that completely covers individual or coalesced lesions (Fig. 2). This cushion, which consists of a dense growth of flexuous conidiophores and their conidia, distinctly separates infection by *C. vanderysti* from that by *C. canescens* or any other bean disease in the country. The symptoms caused by *C. canescens* in Colombia are the same as those described by Zaumeyer and Thomas (5). *C. vanderysti* and *C. canescens* have been seen on rare occasions in the same bean field or on the same plants, and the symptoms were always characteristic of the separate species.

Culture and pathogenicity of the fungus.—It has not been possible to culture *C. vanderysti* on any natural or synthetic medium. Inoculations with spores and mycelium from diseased bean leaves onto healthy leaves have produced atypical lesions in the greenhouse. Within these lesions conidia quite typical of the species developed.

Fig. 2. The lower surface of a bean leaf, showing the cushions of conidiophores and conidia that completely cover the lesions of *C. vanderysti*. This sign is very characteristic of mancha gris.

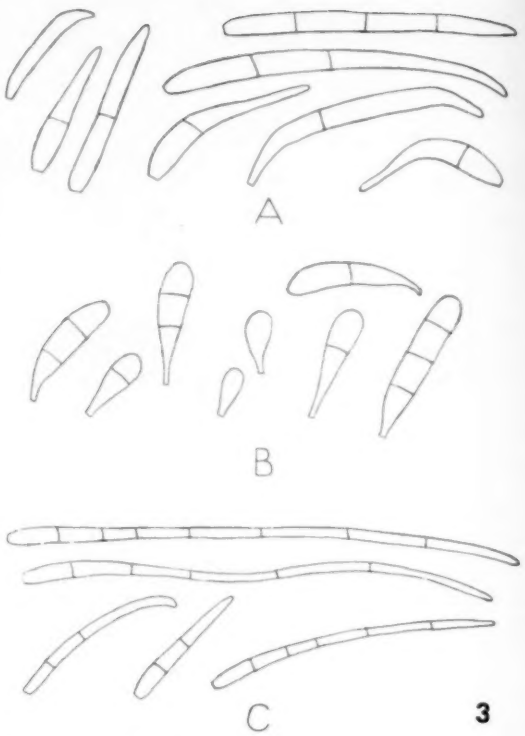

Fig. 3. Morphology of the conidia (A) from the upper surface and (B) the lower surface of a bean leaf infected by *Cercospora vanderysti*, and (C) from a leaf infected by *C. canescens*.

Morphology of the fungus.—The conidia produced by *C. vanderysti* on the upper and lower surfaces of bean leaves in the field are quite distinct. The characteristics of each conidial type are shown in Fig. 3, in comparison with the conidia of *C. canescens* as it occurs in Colombia. An amended description of *C. vanderysti*, including its characteristics on *Phaseolus vulgaris*, is given below:

> Leaf lesions pale green, usually angular when small, limited by veinlets, 2–5 mm in diameter, later enlarging and coalescing to cover most of leaf blade; fruiting in effuse, appressed tomentose, dingy-gray to mouse-colored hypophyllous layers and in indistinct, grayish-white, powdery epiphyllous layers; stromata lacking; hypophyllous conidiophores in long, dense, plainly branched, sinuous or flexuous, widely divergent fascicles, 4–5.5 × 50–300 μ, commonly 160–220 μ, length difficult to determine because of flexuous habit; epiphyllous conidiophores in short, compact, erect, sinuous, little- or unbranched fascicles, 4–5.5×30–115 μ; all conidiophores medium yellowish-olivaceous to olivaceous-brown, uniform in color, multiseptate, irregular in width, not constricted at septa, 1-multigeniculate, tip obtuse to short conically truncate; epiphyllous conidia hyaline, cylindric to obclavate, straight to distinctly curved or occasionally sharply bent, wall slightly undulating, 0–3-septate, mostly 1-septate, base subtruncate to long obconically truncate, tip

obtuse to subacute, 4–5×16–56 μ; hypophyllous conidia hyaline, cylindric to clavate, straight to curved, wall straight, 0–3-septate, mostly 2-septate, base subtruncate to short or long obconically truncate, tip obtuse, 4–5×16–38 μ.

DISTRIBUTION AND SEVERITY OF THE DISEASE.—*C. vanderysti* has a geographical range in Colombia very similar to that of *Isariopsis griseola;* its development appears favored by about the same environmental factors. At altitudes of 1500–2200 m, both pathogens are prevalent on beans every growing season, whereas *C. canescens* is practically unknown. In the Cauca Valley, at an altitude of 1000 m, *C. vanderysti* is rarely seen, and then only during the cooler, more humid seasons. Similarly, *I. griseola* is seen there during the humid, cool growing seasons. *C. canescens* is observed every year in this same region, but prevalence and severity are greatest during warm, dry weather. At such times *C. vanderysti* cannot be found and the prevalence of *I. griseola* is negligible.

No studies have been made of the effect of mancha gris on bean yields in Colombia. The fact that the disease often becomes widespread early in the growing season in the 1500–2200-m zone, resulting in premature defoliation, indicates that it is potentially quite serious at these altitudes. Since 1953, when the disease was first seen, it has become more prevalent and has appeared successively earlier in the growing seasons. In 1957 it was the first disease observed in the bean fields at Medellin, where considerable experimental work is done, and it spread more rapidly than any other disease over a wide variety of bean collections and breeding lines. Some of the collections showed a very high degree of resistance to mancha gris.

ESTACION EXPERIMENTAL AGROPECUARIA "TULIO OSPINA"
MEDELLIN, COLOMBIA

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CONTROL OF ROOT-KNOT NEMATODES AFFECTING PORTO RICO SWEETPOTATOES[1]

L. W. Nielsen and J. N. Sasser

SUMMARY

Various soil nematocide treatments were investigated for 3 growing seasons in relation to Porto Rico sweetpotato production and quality. *Meloidogyne incognita acrita* was the principal nematode species present in the soils studied. D-D and EDB (W-85) were compared at different dosages in row applications with and without vermiculite as a carrier. Tests were performed in soils classified as Norfolk sand, Norfolk sandy loam, and Portsmouth loam. The nematocide treatments effectively killed nematode larvae and increased the yield and quality of sweetpotato roots. Maximum nematode control and yield increases, and fewest cracked roots, were obtained with D-D and EDB at the recommended row application dosages of 10 gal. and 2¼ gal. per acre, respectively. In some tests vermiculite as a carrier tended to improve the effectiveness of the nematocides. This response was not consistent in the various soils from year to year. Soil type also influenced plant response to D-D and EDB. Both fumigants reduced the numbers of nematodes in the 3 soils, but EDB tended to be phytotoxic at 2 times the recommended dosage, in the heavy soil particularly. Reduction in nematode population was correlated with a reduction in the percentage of cracked roots.

Root-knot nematodes (*Meloidogyne* spp.) are widely distributed in the North Carolina sweetpotato-producing areas. These organisms reduce the productivity of many crops, but their effect on sweetpotato (*Ipomoea batatas* (L.) Law.) has not been clearly understood, nor have the benefits to be derived from nematode control with soil fumigants. An investigation during 3 growing seasons, beginning in 1954, was initiated to study 1) the pathogenesis of the root-knot nematode, *Meloidogyne incognita acrita* Chitwood 1949, to the sweetpotato variety Porto Rico, and 2) the practicability of controlling root-knot of sweetpotatoes through nematocide treatment of infested soils. The pathogenesis phase of the investigation has been reported (5), and an abstract has been published on the control studies (8). This paper describes the control studies and results in detail.

These investigations considered such questions as the necessity of treating all soils, the relative merits of D-D (1,3-dichloropropene; 1,2-dichloropropane) and EDB (Dowfume W-85, 83% 1,2-dibromoethane), nematocide phytotoxicity, the merit of vermiculite as a

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nematocide carrier, and the influence of soil type on nematocide activity.

LITERATURE REVIEW.—Root-knot-nematode infection of sweetpotatoes has been observed to reduce root yields markedly in susceptible varieties (2, 9, 13). Two of these reports (9, 13) indicate that the Porto Rico variety possesses resistance to the pathogen and can be grown in infested soils. Mullin (7) reported that yields of Porto Rico in infested soils were not increased by D-D or EDB.

Root-knot-nematode infection of sweetpotatoes also affects the quality of the roots produced. Elliot (2) illustrated a severely cracked root from an infected crop, but did not mention root cracking in the syndrome of the disease. Poole and Schmidt (9) discussed and illustrated the cracking of infected roots, but did not attribute cracking to nematode infection. With the advent of economical nematocides, it was demonstrated that cracking was reduced by soil fumigation to eliminate root-knot nematodes (6). Artificially infesting pot cultures of sweetpotatoes with root-knot nematodes resulted in a cracking of enlarging roots (7). It has been pointed out that sweetpotato root cracking may also be incited by factors other than nematodes (5, 7).

Vermiculite, used as a carrier of EDB in greenhouse and field-row tests by Sasser and Nusbaum (10), gave satisfactory control of root-knot on tobacco and tomatoes. Winstead, Wells, and Sasser (14) obtained satisfactory control of root-knot on vegetables with D-D or EDB adsorbed on vermiculite and applied in the row.

Organic matter, other soil colloids, and soil compaction influence nematocide diffusion within the soil. Organic matter in the soil markedly influences the diffusion and adsorption of D-D and EDB (11, 12), affecting the pattern of nematode kill (3); more adsorbed nematocide is retained by soils rich in organic matter (11). Increasing soil compaction reduces gaseous diffusion (3, 4), so that nematocides are more effective in porous, sandy soils than in clays or soils rich in organic matter (1).

MATERIALS AND METHODS.—All experiments were conducted under field conditions. In preliminary tests, soils of unknown infestation were treated, but the more critical experiments were conducted in soils infested with *M. incognita acrita*.

For tests conducted in 1955 and 1956, the nematocides were applied May 16 and 22, and plants of the Porto Rico variety, used in all tests, were set by the cooperating grower June 6 and 7, respectively. Plants were set at about 12-in. intervals in rows 3½ ft. apart. Nematocide dosage was that recommended for row application for other crops: D-D, 10 gal./A.; EDB, 2¼ gal./A. active ingredient. In a few treatments EDB was applied broadcast at 4½ gal./A. In the comprehensive tests each nematocide was also applied at ½ (½x) and double (2x) the recommended row dosage (x). The nematocides were applied both as liquids and adsorbed on vermiculite.

The nematocides and vermiculite were mixed the day preceding application and stored in sealed fiber containers. Vermiculite was used at 60 lb. dry weight per A., and predetermined volumes of each nematocide were mixed manually with the carrier.

Most treatments were applied with row equipment. Liquids were applied with a single-delivery-tube gravity-flow appliance attached to a small handplow. A single-tube fertilizer distributor was used for applying the vermiculite mixtures. Both machines delivered the nematocides to the bottom of previously opened rows—6–8 in. deep. Two to 3 in. of loose soil entrapped the fumigants immediately after delivery. The rows were then ridged 15–18 in. high with tractor-drawn plows.

Two plot designs were used: paired plots in preliminary tests; factorial designs in later experiments. Various data were recorded, but greatest emphasis was placed on total yield of roots of U. S. No. 1 size, the percentage of these roots with growth cracks, and the nematode populations in the soils following treatment.

EXPERIMENTAL RESULTS.—The preliminary tests were designed to evaluate recommended nematocides for increasing the productivity of sweetpotatoes, and to determine whether all soils should be treated. The cooperating growers were advised to treat one half of the soil, irrespective of previous nematode history. Six fields were treated in 3 counties, all with EDB, broadcast in 2 fields and applied as row treatments in the others. Composite soil samples, taken from each of 5–10 paired plots 50 ft. long at each location in late July, were assayed for parasitic nematodes by the Baermann funnel method. The paired plots were located in treated and untreated soil down the center of each field. Yield and root-cracking data were recorded at harvest.

In one field with broadcast treatment and 2 with row treatments, fumigation increased yields and decreased the incidence of cracking while greatly reducing the nematode populations. In these fields, root-knot nematode larvae were abundant in the non-fumigated soil. In the other 3 locations, few root-knot nematodes were found, cracking was a minor problem, and yields were not improved by fumigation. In one of these latter locations the stunt nematode (*Tylenchorhynchus claytoni* Steiner 1937) was the dominant species in the soil, but no response to fumigation was measured in potato yield from this land, suggesting that this nematode causes little injury to sweetpotatoes. These preliminary tests showed that root-knot nematodes reduce both the productivity and quality of Porto Rico sweetpotatoes, and that treatments are not practical in all sweetpotato soils.

The second year's study was enlarged to compare D-D and EDB at 3 dosages (1/2x, x, and 2x), either as liquids or adsorbed on vermiculite. These treatments were arranged in a factorial design with 4 replications in plots 50 ft. long. In addition, 4 plots received no treatment. The experiment was performed in 2 infested fields about 3 miles apart. Location 1 was predominantly a Norfolk sand; location 2 varied from a Norfolk sandy loam to a Portsmouth loam.

TABLE 1.—*Yields and per cent of roots cracked in Porto Rico sweetpotatoes grown in nematode-infested soil treated with D-D or EDB*

Treatment	Dosage	Lb. of roots		Per cent roots cracked	
		Location 1	Location 2	Location 1	Location 2
D-D liquid	½x [a]	19.7	21.6	17.5	30.5
	x	19.0	37.3	24.5	21.3
	2x	26.8	36.4	36.5	19.0
D-D + vermiculite	½x	24.3	45.4	37.7	20.0
	x	26.5	41.3	40.0	29.8
	2x	25.5	22.8	40.5	30.0
EDB liquid	½x	17.4	27.7	37.3	27.3
	x	23.7	34.7	28.7	27.3
	2x	23.0	33.8	19.3	14.5
EDB + vermiculite	½x	34.0	33.3	24.0	17.3
	x	24.9	25.2	27.3	23.5
	2x	30.8	15.5	14.3	27.5
No treatment		16.6	25.1	70.8	39.8
LSD 5%		11.6	14.8	19.6	21.5
Significant interactions:[b]					
N×V			*	*	
D×V			**		
D×N				*	

[a] x = dosage recommended for row application for other crops: D-D, 10 gal./A.; EDB, 2¼ gal./A.
[b] N = nematocide; V = vermiculite; D = dosage of nematocides. * = significant at 5% level; ** = significant at 1% level.

Striking differences in vine growth were evident on the different plots in late August after a period of drouth. Plants in untreated soil were stunted, with chlorotic foliage. In contrast, plants growing in some treated plots were vigorous, with rich green foliage, and with vines covering the space between rows. At location 1 these differences persisted until harvest—October 12.

Yield, root cracking, and soil nematode data were taken at harvest. Composite soil samples from plots receiving liquid nematocides at normal dosage and from untreated plots were assayed for root-knot nematode populations. The treated and untreated soils at both locations contained large numbers of larvae. Counts were about 2500–4500 per pint of soil. Yields and root-cracking data and significant interactions are summarized in Table 1.

At location 1, some treatments increased the yield of roots over that from untreated soil. When the percentage of cracked roots is eliminated from the yield data, all treatments increased the yield of crack-free roots over that from untreated soil. Largest yields were obtained in those plots receiving the EDB-vermiculite mixtures. The addition of vermiculite to the nematocides resulted in a mean yield of 27.6 pounds per plot, which was more (at 5% significance) than the mean of 21.6 pounds produced by plots treated with the liquid nematocides.

Yield increases were also measured at location 2, which had a more variable soil. Here, the yields tended to increase with the increase in dosage of the liquid nematocides. The addition of vermiculite resulted in lower yields at higher dosages. The magnitude of this interaction (D×V) was highly significant, and suggests that vermiculite increased nematocide toxicity, which reached phytotoxicity at the 2x dosage.

All treatments at both locations reduced root cracking below that in untreated plots. Much of the cracking may have been caused by nematodes, for larvae were numerous in treated soils at harvest time. Variation in these data was large, and no treatment was outstanding.

It was apparent that the several treatments had quite different effects at the two locations, but most were superior to not treating. Two factors were considered of great importance to these differences: type of soil and climate. An attempt was made to measure the influence of soil type on nematocidal action under similar climatic fluctuations. It must be acknowledged that physical environments will differ within different soils exposed to the same precipitation, air temperature, and radiant energy.

The third experiment was located in a field in the Coastal Plain that contained soils classified as Norfolk sand, Norfolk sandy loam, and Portsmouth loam. The most distant plot areas (Norfolk sand and Portsmouth loam) were about 100 yards apart. The Portsmouth loam was 3–5 ft. lower in elevation than the Norfolk sand. Composite soil samples were taken from each plot after planting (all plots received the same fertilizer treatment), and were subjected to routine soil analyses[2] (Table 2).

The trend toward greater concentrations of nutrient elements and organic matter in Portsmouth loam than in Norfolk sand was expected. The Norfolk sandy loam soil was located in an old homesite, which accounts for the greater concentrations of calcium and phosphorus, reflected in pH. Of the several soil factors listed in the table, organic matter was expected to play the most important role in nematocide activity.

[2] The writers are indebted to Mr. Charles Welch and the personnel of the Soil Testing Division, N. C. Department of Agriculture, Raleigh, for this service.

TABLE 2.—*Mean concentration of major nutrient elements, organic matter, and hydrogen-ions in 3 experimental soils*

Soil	Potassium (me)	Calcium (me)	Phosphorus (ppm)	Organic matter (%)	pH
Norfolk sand	.195	0.43	82.0	0.96	5.12
Norfolk sandy loam	.246	3.69	125.6	1.44	6.01
Portsmouth loam	.277	1.60	86.9	2.34	4.99

Treatments similar to those used in the preceding test were arranged in a factorial design and applied to the three soils. A vermiculite control was included. There were 16 treatments replicated 4 times in plots 35 ft. long.

Data were recorded on yield, root cracking, plant survival, and nematode populations (mid-July and at harvest).

Plant survival.—Nematocide treatments influenced plant survival in Norfolk sand and Portsmouth loam. In Norfolk sand there was better survival following treatments with D-D than with EDB (24.3 vs. 21.1 plants per plot). In Portsmouth loam there were fewer plants when the nematocides were adsorbed on vermiculite than when used as liquids (22.4 vs. 25.9 per plot).

Dosage was the most significant treatment variable, whether applied as liquid or adsorbed on vermiculite. Table 3 treats the three soils as individual experiments, presenting data for nematode larvae in the soil at harvest, yield of roots, and proportion of roots with cracks.

Nematode populations.—Soil populations of *M. incognita acrita* larvae were very different in mid-July and mid-October. There were too few larvae in mid-July to make comparisons among plots. At harvest, the numbers of larvae were different in the three soils, with the smallest number in Portsmouth loam. Nematocide treatments also modified the number of larvae present at harvest. The fewest larvae (Table 3) were in those plots of each soil receiving the 2x dosage. There was no statistical difference in numbers following the 1x and 2x dosages, but there was a tendency for better control in Norfolk sand and Norfolk sandy loam soils at the heavier dosage. This trend was not evident in Portsmouth loam.

Yield of potatoes.—Both nematocides effected large increases in yield at the higher dosages in the lighter soils, but not in Portsmouth loam (Table 3). Recommended dosages (1x) of both nematocides were as effective as the 2x dosage. The failure to obtain a yield response in Portsmouth loam is possibly associated with its greater water-holding capacity and poorer natural drainage. There was periodic drouth during the growing season.

Although dosage differences yielded no difference in Portsmouth loam, there was a yield response in favor of D-D. Plots treated with D-D averaged 23.4 lb. per plot, whereas those treated with EDB averaged 18.0 lb. per plot. This difference is discussed further below.

Root cracking was reduced in the three soils by controlling nematodes (Table 3). The magnitude of reduction in cracking was similar in each soil. Maximum reduction was achieved with the recommended dosage (1x). It is evident that nematodes were a production problem in the Portsmouth loam. The cracking in plots treated with the 2x dosage may be due to nonuniform application of nematocides or cracking characterized as physiological and commonly associated with variations in soil nutrients and moisture during root enlargement.

To measure the influence of soil type on behavior of nematocide treatments, data on surviving plants, nematode population, yield of marketable-size roots, and per cent of roots cracked were combined for statistical analyses (Table 4).

All treatment variables except nematocides were effective as measured by one or more categories of data for the three soils. Dosage of nematocide application was the most important treatment variable, as pointed out above. Soil type influenced nematode population and yield of potatoes. More nematode larvae were found in Norfolk sand and Norfolk sandy loam, suggesting that these soils favor nematode multiplication more than does the heavier Portsmouth loam. The greater yield in Portsmouth loam may be due, in part, to fewer nematodes, but, as mentioned earlier, available nutrients and, possibly, moisture also contributed. Using vermiculite as the nematocide carrier reduced the number of surviving plants. This reduction was slight in Norfolk sand and Norfolk sandy loam, but significant in Portsmouth loam.

TABLE 3.—*Influence of nematocide dosage on root-knot nematode larvae at harvest, yield of marketable-size roots, and cracked roots in 3 soils*

Dosage [a]	Norfolk sand			Norfolk sandy loam			Portsmouth loam		
	Larvae [b]	Yield [c] (lb.)	Cracked roots (%)	Larvae [b]	Yield [c] (lb.)	Cracked roots (%)	Larvae [b]	Yield [c] (lb.)	Cracked roots (%)
None	675.2	6.8	16.5	646.4	6.6	19.1	388.8	22.6	19.8
½x	811.2	12.1	11.2	353.6	12.1	13.3	312.0	20.7	10.4
1x	433.6	15.1	4.0	430.4	15.0	3.0	14.4	20.7	4.1
2x	161.6	16.3	1.4	105.6	18.9	3.3	36.8	18.9	4.2
LSD 5%	332.8	3.9	9.0	350.4	4.0	10.0	211.2	N.S.	7.5

[a] x = dosage recommended for row application for other crops: D-D, 10 gal./A.; EDB, 2¼ gal./A.
[b] Number of larvae per pint of soil.
[c] Yield includes both cracked and uncracked roots of U. S. No. 1 size.

TABLE 4.—*Significant variables and variable interactions of nematocide treatments in 3 soils as measured by surviving plants, nematode populations at harvest, yields of roots, and percentage of cracked roots.*

Treatment variables and interactions	Surviving plants	Relative significance [a] Nematodes	Yields	Cracked roots
Dosage (D)	ns	**	**	**
Nematocides (N)	ns	ns	ns	ns
Soils (S)	*	**[b]	*	ns
Vermiculite (V)	*	ns	ns	ns
D×N	ns	*	*	ns
D×S	ns	ns	**	ns
N×S	**	ns	*	ns
D×V×S	ns	*	ns	ns
D×N×V	ns	ns	ns	*
D×N×V×S	ns	*	ns	*

[a] * = significant at 5% level; ** = significant at 1% level; ns = not significant.

[b] The identity of replications was lost, and this F value could not be computed. In comparison with other mean squares, the value appeared to be highly significant.

There were several significant interactions between the treatment variables. Only the less complex, first-order interactions are discussed. Nematocides did not differ when compared one with the other, but did differ when the interactions are considered. The interaction N×S was significant for surviving plants and yield of potatoes. Plant survival in plots treated with EDB varied from a low of 21.1 plants, in Norfolk sand, to a high of 25.2 plants per plot, in Portsmouth loam; whereas, for D-D, surviving plants varied from 24.2 to 25.0 per plot in the three soils. These differences in surviving plants were not reflected in yields of potatoes (Fig. 1A). Plots treated with the two nematocides produced similar yields in the sandy soils, but yield was depressed by EDB in Portsmouth loam. The colloidal systems of the latter soil possibly adsorbed and retained EDB after the plants were set. As the gas was slowly released, the concentration was at a sublethal dosage, yet sufficiently concentrated to impair plant growth.

Two other interactions involving dosage (D×S and D×N) also influenced potato yield. Dosage × soil was highly significant, a relationship illustrated in Fig. 1B. In Norfolk sand and Norfolk sandy loam, the yield of roots was positively correlated to dosage of nematocide application. In contrast, the response in Portsmouth loam was negatively correlated to dosage, suggesting phytotoxicity. This phytotoxic effect was due to the performance of EDB, as shown in Fig. 1A and C. As the test soil became richer in organic matter, the 2x dosage of EDB tended to become phytotoxic, reaching its fullest expression in the heavy soil. Other soil factors—compaction, water content, etc.—may have contributed to this response.

DISCUSSION.—Adsorption of nematocides on vermiculite makes possible their application with a fertilizer distributor. Most fertilizer applicators are geared to the wheels of the machine on which they are mounted, largely eliminating dosage variations from changes in speed. With gravity-flow liquid applicators, however, the speed at which the equipment moves alters the rate of application. This disadvantage may justify the additional cost of vermiculite and labor for mixing, and vermiculite provided other advantages in some tests.

However, vermiculite tended to increase the nematocide toxicity. One season, maximum yields were experienced (Table 1, location 2) with both nematocides at ½x dosage on vermiculite. At higher dosages the yields declined, suggesting phytotoxicity. This increased toxicity was also evident in Portsmouth loam, where plant survival was reduced in plots treated with vermiculite mixtures. Vermiculite also contributed to a number of the significant interactions involving nema-

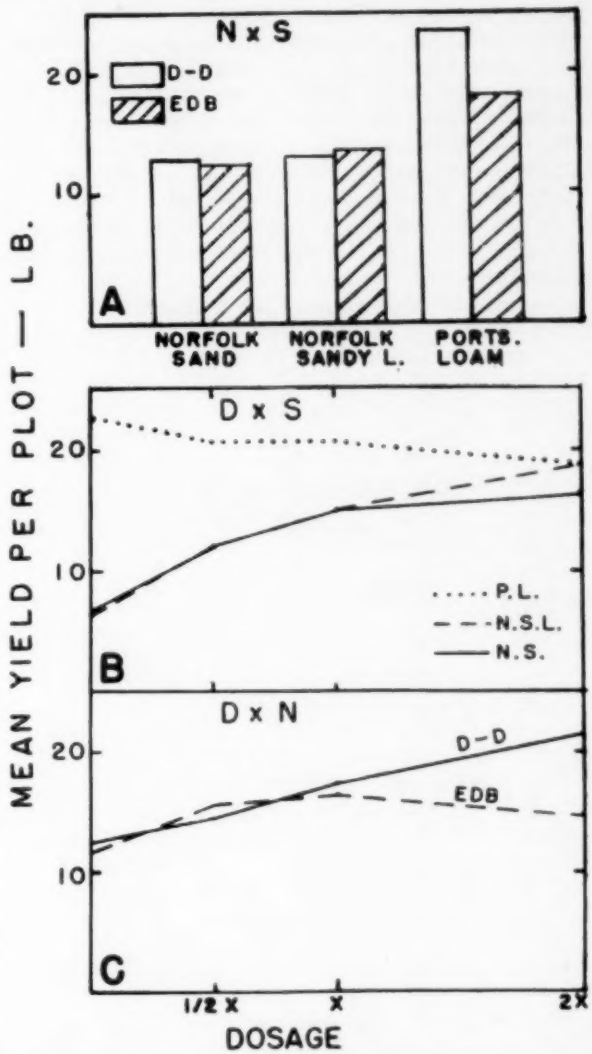

Fig. 1. Graphic illustrations of significant first-order interactions as measured by yield of potatoes. **A)** Nematocide × soils—yield of sweetpotatoes following treatments of Norfolk sand, Norfolk sandy loam, and Portsmouth loam with D-D and EDB. **B)** Dosage × soils—yield of roots is positively correlated with dosage in Norfolk sand (N.S.) and Norfolk sandy loam (N.S.L.), but negatively correlated in Portsmouth loam (P.L.). **C)** Dosage × nematocide—EDB tended to reduce yields at the higher dosage.

tocide dosage. It is not known how vermiculite influences the nematocides, but it seems reasonable to assume that it slows the rate of release of gas to the soil system.

Both D-D and EDB were effective in destroying *M. incognita acrita* larvae in infested soils, resulting in large increases in plant productivity and root quality. Recommended dosages, either broadcast or in the row, were adequate for practical control. There was a tendency, in heavier soils, for EDB to be phytotoxic if plantings were made about 2 weeks after application. It is not known whether a longer aeration would eliminate this phytotoxicity.

Nematode populations in the soil rapidly increase on sweetpotatoes following soil treatment with D-D (5), and at the end of the growing season the populations in treated and untreated soils may be very similar. The same was true in these studies except for EDB in Portsmouth loam. Evidently, greatest root-knot nematode injury to the sweetpotato plant occurs early in the growing season. Perhaps by reducing the soil larvae population, the nematocides permit young plants to become so well established that they become capable of supporting the ever-increasing nematode population without serious root injury. If sweetpotatoes are in a cropping sequence with other high-value root-knot-susceptible crops, infested soils will have to be treated annually.

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PECTOLYTIC ENZYMES OF PSEUDOMONAS MARGINALIS AND THEIR EFFECTS ON LETTUCE [1]

M. J. Ceponis and B. A. Friedman

SUMMARY

Soft rot, vascular browning, and russet spotting were produced on lettuce leaves with sterile filtrates of *Pseudomonas marginalis* cultures. Strong protopectinase and pectin depolymerase activity were found in filtrates with pH optima about 7.5–8.5. The activity of these enzymes was reduced sharply by heating 20 minutes above 50°C. Pectinmethylesterase activity was weak in the culture filtrates. A polygalacturonase forming monogalacturonic acid could not be detected.

Pseudomonas marginalis (Brown) Stevens causes a soft rot of head lettuce (*Lactuca sativa* L.). In addition, vascular browning and russet spotting are observed occasionally on naturally infected heads (8). The present paper reports on the types of pectolytic enzymes produced in culture by *Ps. marginalis* and gives additional details on the production of vascular browning and russet spotting with sterile filtrates. A preliminary report has appeared (2).

MATERIALS AND METHODS.—A virulent culture of *Ps. marginalis*, described previously (7), was grown in lettuce broth at 25°C. usually for 4 days. The culture medium was prepared by blending 500 g of head lettuce with water containing 0.2% $MgSO_4 \cdot 7H_2O$ and 0.1% K_2HPO_4, boiling 5 minutes, and straining through cotton. The volume was brought up to 1 liter by adding the mineral-salt solution and 10 g L-asparagine. The pH was adjusted to 7.0 before autoclave sterilization. After the incubation period the culture broth was centrifuged to remove bacterial cells, and sterile filtrates were prepared from the supernatant fluid by passage through Seitz pads. The filtrates were used without concentration. To produce vascular browning in Romaine lettuce, washed leaves were placed in a beaker containing 50 ml of filtrate with 100 ppm of oxytetracycline, and briefly exposed to a vacuum of about 60 mm of mercury to facilitate filtrate uptake. The leaves were allowed to stand in the filtrate up to 20 hours at 22–29°C. To produce russet spotting, Romaine and head-lettuce leaves were either spotted with or momentarily dipped in filtrate containing the antibiotic and held overnight in moist chambers at 22–29°C. In controls, active filtrates were replaced by boiled or autoclaved filtrates, sterile broth, or tap water.

For tests of the rate of maceration (PP activity),[2] cores 13 mm in diameter were bored out of the butts of heads of lettuce and cut by microtome into slices 400 μ thick. The slices were placed in 9–35 ml of filtrate at 21–26°C. and observations were made on the time required for a blunt needle (diameter 0.032 in.) with a total weight of 17 g to penetrate the softened tissues. DP activity was measured in 2-ml Ostwald viscosimeters at 30°C with reaction mixtures consisting of 9 parts of 0.56% low methoxyl citrus pectin solution (Matheson, Coleman and Bell Co.) to 1 part of culture filtrate containing 0.005*M* $CaCl_2$. Filtrates were heated 20 minutes at 30–70°C and cooled in ice-water, and the effects of heating on maceration and viscosity were observed. To detect PG, the liberation of reducing groups from 0.5% sodium pectate solution (Nutritional Biochemicals Corp.) by 10 ml of filtrate in 100 ml of reaction mixture was determined by the hypoiodite method (12), and the appearance of monogalacturonic acid was tested by paper chromatography (5). To test PME activity, 10 ml of filtrate containing 0.005*M* $CaCl_2$ was added to 90 ml of citrus pectin solution (Nutritional Biochemicals Corp.) to give a final concentration of 1.1% pectin, and the formation of carboxyl groups during de-esterification at 30°C was determined by titration with 0.02*N* NaOH (16, 18, 23). The pH of the substrates were adjusted with 0.1*N* NaOH, or in some cases with 0.1*N* HCl and checked by glass electrode.

RESULTS.—*Vascular browning and russet spotting.*—Heads of lettuce infected naturally with bacterial soft rot caused by *Ps. marginalis* occasionally develop discolored veins and russet spotting some distance from the site of the active soft rot (Fig. 1-AB). Upon microscopic examination or culture on nutrient agar, the lesions were observed to be sterile or to contain relatively few bacteria. In 2 experiments, 24 Romaine lettuce leaves standing in sterile culture filtrates developed moderate to severe vascular discolorations in the midribs and lateral veins of all leaves within 17 hours. In addition, some severely affected leaves had a pronounced soft rot of the midrib or interveinal tissues (Fig. 1-C). In 2 tests, 14 leaves of each of Romaine and head lettuce were spotted with or dipped in active filtrate and held 17–20 hours in moist chambers. All developed russet spotting (Fig. 1-D). In most instances the dipped leaves also exhibited vein browning and soft rot. Bacteria were not observed in the lesions. Leaves treated with autoclaved or boiled filtrate, sterile broth, or tap water developed no lesions.

Enzyme assays.—In the maceration tests (PP activity), some variation was found in the time required for the weighted needle to penetrate the lettuce tissue slices in a given experiment. However, this method was found to be more objective than methods in which tissue slices were pulled apart, lifted by forceps, or

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[2] The following symbols are used to designate pectolytic enzymes: PP (protopectinase); DP (pectin depolymerase); PG (polygalacturonase); PME (pectinmethylesterase).

teased with needles. Maceration occurred over a wide pH range, with optimum near 8.5 (Fig. 2-A). Heating the filtrate above 50°C for 20 minutes caused a marked decrease in its macerating activity at pH 8.0 (Fig. 2-B). The viscosity of 0.5% pectin solutions was rapidly reduced in the pH range 6.9–9.0, indicating high DP activity (Fig. 2-A), whereas heating filtrates above 50°C markedly reduced activity (Fig. 2-B). The data in Fig. 2 are from representative experiments.

The increase in reducing groups upon the addition of filtrate to sodium pectate solutions was found to be small (Table 1). Preliminary tests showed the pH optimum to be in the range of 8–9. In 5 different tests at or near pH 8.0, no more than 1.7 ml of 0.05*N* iodine in 100-ml samples was reduced in 20 hours by 10 ml of active filtrate. Monogalacturonic acid production in reaction mixtures could not be demonstrated chromatographically at the end of 24, 48, or 72 hours, whereas controls of monogalacturonic acid or of a reaction mixture containing commercial PG developed the characteristic spot of the acid on the chromatogram.

With a modification of Winstead and Walker's method of PME assay (23), averages of 0.082, 0.091, and 0.106 mg of methoxyl groups per ml of filtrate were removed in 4–5 hours at pH 8. A preliminary test indicated practically complete inactivation of the enzyme at pH 4.5. Results were negative or inconclusive with other assay methods for PME (16, 18).

Discussion.—In slimy soft rot of lettuce caused by *Ps. marginalis*, vascular browning and russet spotting are minor and observed only occasionally. Their production on head lettuce leaves by crude enzyme filtrates of *Ps. marginalis* may account for the manner in which similar symptoms develop in naturally infected lettuce heads in the apparent absence of bacteria. The nature of the mechanism causing vascular

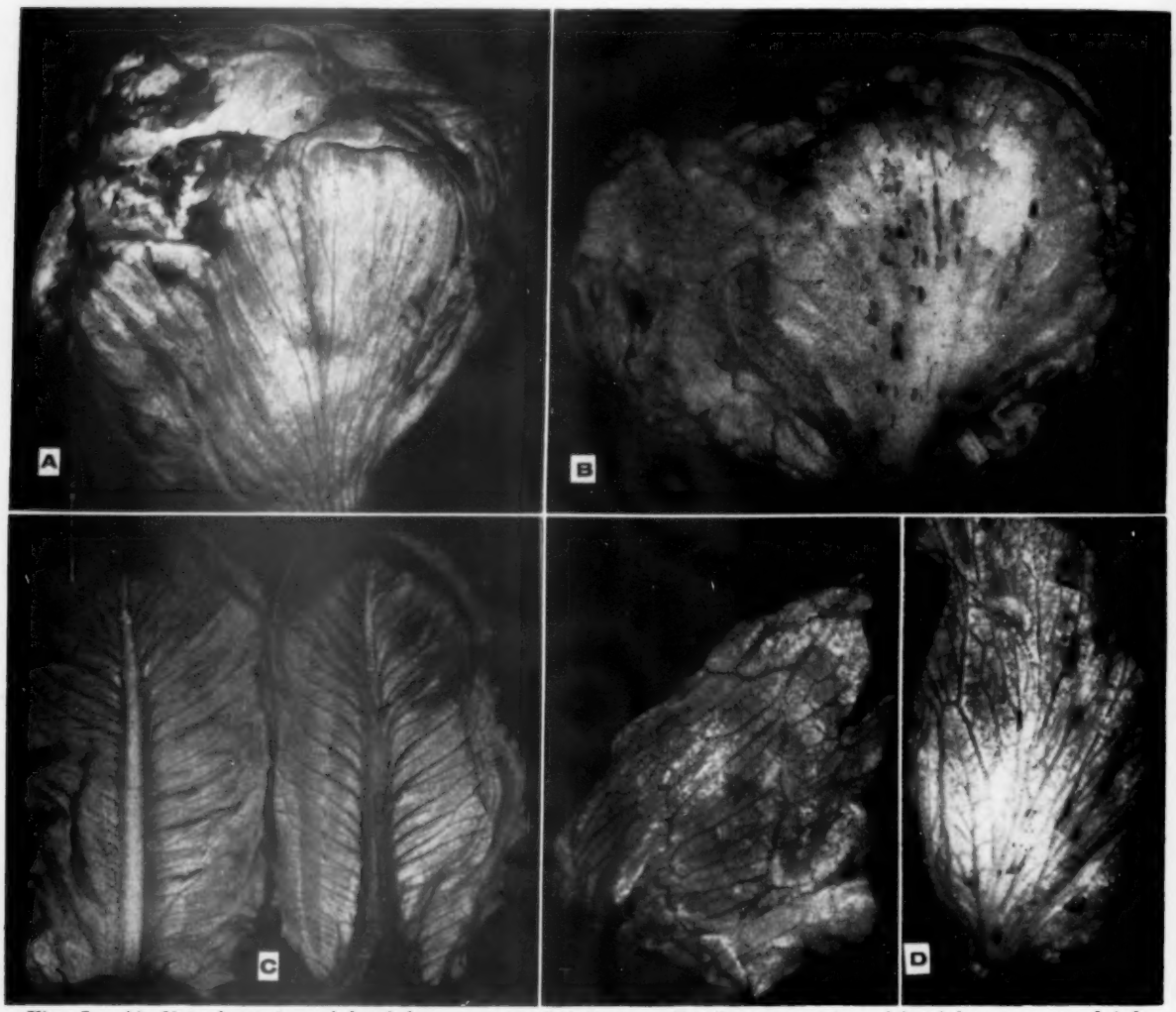

Fig. 1. **A)** Vein browning of head lettuce, natural infection. **B)** Russet spotting of head lettuce, natural infection. **C)** Midrib and vein browning of Romaine lettuce leaf caused by active filtrate of *Ps. marginalis* culture, on right. This leaf also shows midrib soft rot at base. Control leaf treated with boiled filtrate, on left.. **D)** Russet spotting of head lettuce leaf treated with active filtrate, on right. Conrol leaf treated with boiled filtrate, on left.

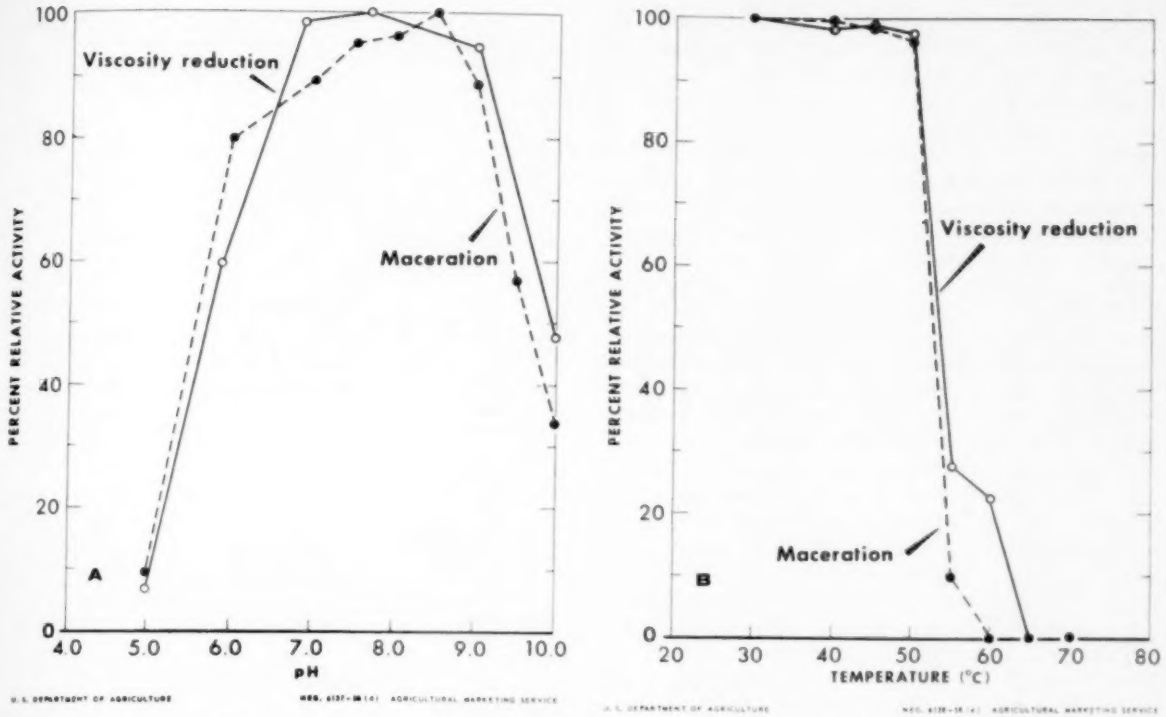

Fig. 2. Maceration of lettuce butt tissue slices (PP activity) and viscosity reduction of pectin solutions (DP activity) by culture filtrates of *Ps. marginalis*. **A)** Effect of pH. **B)** Effect of heat on filtrates at pH 8.0.

discoloration and russeting was not studied, but several hypotheses have been advanced to account for vascular discoloration by fungi and bacteria (9, 10, 22). As reported previously, other types of russet spotting are caused by viruses or physiological factors (8).

The enzyme causing maceration of plant tissue is considered generally to be PP, but the existence of this enzyme has not been demonstrated conclusively (13, p. 158-166). The close similarity or possible identity of PP and DP of *Erwinia aroideae* has been reported (24). The optimum pH for PP activity of *Ps. marginalis* filtrates is about 8.5. Preparations from an unidentified *Pseudomonas* species and *Ps. syringae* were most active at pH 8.0–9.0 in potato maceration (15), and optima ranging from pH 6.5–9.6 were reported for *E. aroideae*, *E. atroseptica*, and *E. carotovora* on various vegetables (1, 4, 6, 24). In the present study the lowest temperature at which PP activity was completely inhibited was not determined, but activity was sharply reduced after heating 20 minutes above 50°C. PP activity of *Erwinia* spp. was reported to be inactivated above 60–65° by the use of different plant tissues and heating times (1, 24).

TABLE 1.—*Production of reducing groups from 0.5% sodium pectate solution by active filtrates of Pseudomonas marginalis cultures*

pH of reaction mixture	Reaction time (hours)	0.05*N* *iodine* reduced[a] (ml)
7.8	3.5	0.64
	20	1.21
8.0	3–3.5	0.49, 0.73, 1.07
	19–21	1.05, 1.42, 1.70
8.5	3	0.41
	6	0.79
	21.5	1.41

[a] Iodine required to reduce 100 ml of reaction mixture having a filtrate-substrate ratio of 1:9. Each figure represents 1 assay.

PG and DP have the capacity to reduce the viscosity of pectin and pectate solutions and to release reducing groups. The limited hydrolysis of the pectate chain by DP, in contrast to its complete hydrolysis to monogalacturonic acid by PG, is regarded by some to indicate DP is a size-specific PG (13). The term DP has been used in recent reports (4, 24), but some workers have given preference to the terms γ-pectin glycosidase (20) or endopolymethylgalacturonase II (3). In the present study, *Ps. marginalis* cultures grown 3 weeks in pectate-mineral salt medium at pH 8.0 degraded all the pectate so that no precipitate formed when calcium ions were added to the culture filtrate. Oxford reported similar results with *Ps. marginalis*, and suggested the presence of PG (19). In the present study, however, only DP activity was demonstrated in *Ps. marginalis* filtrates, since no monogalacturonic acid could be detected chromatographically, despite a filtrate-substrate ratio of 1:3 and long reaction times. Smith reported 2 of 3 strains of *Ps. marginalis* failed to produce DP (γ-pectin glycosidase) and 1 of 3 strains formed monogalacturonic acid in culture. This

worker discussed the conflicting results of earlier work and noted the characteristic variability in pectolytic enzyme production by the genus *Pseudomonas*, which was attributed to strain variations and to the use of stock, inactive cultures (20, 21). In some cases, the identity of cultures may account for the differences reported in the literature, especially with regard to cultures of *Pseudomonas*. *Ps. marginalis* DP activity in the reduction of viscosity of pectin solutions was found to have a broad range around pH 8.0, with a marked decline in that activity after heating above 50°C. Reduction of viscosity by *Ps. marginalis* was reported to be more rapid at pH 7.5 than at lower concentrations (20). The pH optimum of *E. aroideae* in this respect was reported to be around 9.0, and almost complete enzyme inactivation at pH 8.3 occurred after heating 10 minutes at 62.5–65° (24).

Under the experimental conditions tested, PME activity was found to be slight in *Ps. marginalis* culture filtrates. In 3 tests, an average of 0.093 mg of methoxyl groups was removed per ml of filtrate. Comparable activities of 3.014 and 1.912 were reported for *Ps. solanacearum* and *Botrytis cinerea*, respectively (23). The production of PME by *E. carotovora* (14), *Ps. prunicola* (17), and *Ps. solanacearum* (11) has been reported. On the other hand, little or no PME activity has been reported for *Erwinia* spp. and *Ps. marginalis* (4, 20, 24).

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PECTOLYTIC AND CELLULYTIC ENZYMES PRODUCED BY RHIZOPUS STOLONIFER [1]

D. N. Srivastava, [2] E. Echandi, [3] and J. C. Walker

SUMMARY

The enzymes of the pectinase complex produced by *Rhizopus stolonifer* were found to be polygalacturonase and pectin methyl esterase. There were indications of considerable pectin depolymerase activity in the extract of rotted sweet potatoes. The pectolytic activity of the extract was much higher than that of any of the culture filtrates. Optimum pH for the activity of each of the pectolytic enzymes was 4.5. A small amount of cellulase activity was also noticed in the extract of rotted sweet potatoes.

In connection with studies of the *Rhizopus* soft rot of sweet potato it became important to inquire into the nature of the host-tissue-macerating enzymes produced by the causal fungus, *Rhizopus stolonifer* (Ehr. ex Fr.) Lind. The first report on this subject is that by Harter and Weimer (3), who showed that pectolytic enzymes were concerned. For detailed information on pectin chemistry and the pectolytic enzymes, the reader is referred to the book by Kertesz (5); a brief definition of pectic substances and the various pectolytic enzymes known is presented here.

The pectic materials in plants are contained largely in cell walls. Pectic substances are composed largely of residues of monogalacturonic acid linked, presumably, in linear chains of various lengths through positions 1 and 4. The carboxyl groups of the chain may be esterified with methyl alcohol or they may form salts with various cations. Pectic acid is the term applied to pectic substances composed of colloidal polygalacturonic acid free from methyl ester groups. Pectic acid forms salts with various cations to form pectates, e.g., sodium polypectate. When a small proportion of the carboxyl groups are esterified, the substance is referred to as pectinic acid. When a relatively high proportion of the carboxyl groups are esterified, the substance is known as pectin. The water-insoluble parent substance in the primary wall is referred to as protopectin. The nature of the latter is still poorly defined.

PME (pectin methyl esterase) is the enzyme that catalyzes the hydrolysis of the methyl ester groups, forming methanol and pectinic or pectic acids. It occurs in higher plants and is known to be produced by numerous fungi and bacteria. PG (polygalacturonase) is a distinct enzyme, so far known to be formed only by microorganisms. This catalyzes the hydrolysis of glycosidic bonds of the pectin chain, breaking the latter down to polygalacturonic acids of decreasing molecular weight, eventually forming monogalacturonic acid. A third enzyme system, most recently discovered and least completely understood, is DP (pectin depolymerase). This also is produced by fungi and bacteria and has been found in tomato fruits. DP also catalyzes the hydrolysis of glycosidic bonds of the pectin chain, but differs from PG in that the hydrolysis it causes does not proceed to the point where monogalacturonic acid is formed. It should be stressed here that PME, DP, and PG, as at present defined, probably represent groups of closely related enzymes rather than single enzymes. Much more fundamental work is needed in this enzyme group before their relations and activities are fully understood.

METHODS AND MATERIALS.—The culture of *Rhizopus stolonifer* used in this investigation was a (+) strain, secured from K. B. Raper, labeled as NRRL 1477. The following media were used: 1) wheat bran medium—commercial wheat bran, 25 g; distilled water, 40 ml. 2) sweet potato broth—peeled sweet potatoes, 500 g; distilled water, 1000 ml. 3) Fernando's medium—$MgSO_4 \cdot 7H_2O$, 5 g; KH_2PO_4, 6.8 g; asparagine, 5 g; glucose, 15 g; distilled water, 1000 ml.

Cultures were grown in 32-oz. pharmaceutical bottles; 250 ml of the liquid medium or 25 g of wheat bran was used per bottle. Culture filtrates were collected after 3–5 days. To each bottle 4–5 drops of toluene were added, and the mycelium was allowed to autolyze for 3 hours. In the case of the liquid media, the broth was centrifuged and the clear supernatant was frozen for use. In the case of the wheat bran, 200 ml of distilled water was added to each bottle before toluene was added. The extract was filtered through cheesecloth and centrifuged, and the supernatant was frozen. Extracts of rotting sweet potatoes were collected by gently pressing the decayed potatoes. The juice thus extracted was centrifuged and frozen.

Crude enzyme preparations were made by precipitating 1000 ml of highly cleared extracts of rotted sweet potatoes with 4 times the volume of 95% ethanol. Both extract and ethanol were cooled to nearly 0°C before mixing. The precipitate was allowed to sediment at 4°C, and later centrifuged, dried, and stored at subzero temperature. Extract from healthy sweet potatoes was prepared by mashing 500 g of grated tissue with 500 ml of water in a Waring Blendor. The pulp was filtered, the filtrate centrifuged, and the supernatant frozen.

For maceration assays, carrot root sections 1 cm

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square and 200 μ thick were cut on a hand microtome. Fifteen ml of the filtrate or the enzyme solution was used for each maceration test. The carrot blocks were inserted in the filtrate, and the end point of the activity was determined as the number of minutes after which the blocks could not be lifted by the edge with a forceps. PME activity was determined by the procedure described by Winstead and Walker (6), and expressed as mg methoxyl groups removed at 3 hours by 1 ml of the filtrate at 30°C. The activity of the hydrolytic enzymes PG and DP was determined first by the viscosity reduction test, and second by the reducing-group test. For the viscosity test, 5 ml of a 2:3:10 mixture of the filtrate, 0.5*M* acetate buffer, and 1.5% pectin solution was placed in an Ostwald viscosimeter and the rate of flow recorded in seconds. The results were calculated according to the formula given by Kertesz (5, p. 382) and expressed as per cent reduction in viscosity at different intervals. The reducing groups resulting from the activity of the filtrate on buffered solution (0.5%) of sodium polypectate were measured by the procedure described by Winstead and Walker (6). The results obtained were expressed as ml of 0.1*N* iodine solution required to be reduced by 5 ml of the reaction mixture, 45:4:1 of 0.5% solution of sodium polypectate, filtrate, and 0.5*M* acetate buffer. Both of the above tests were carried out at 30°C.

The presence of monogalacturonic acid was determined by Ehrlich's basic lead acetate test described by Kertesz (5), and by paper chromatography. For the former, 2 ml of a freshly filtered 25% solution of basic lead acetate was mixed with 5 ml of buffered enzyme and sodium polypectate mixture after digestion for 24 hours at 30°C. The mixture was heated 5 minutes in boiling water and the color of the precipitate recorded. For the ascending paper chromatography test, Whatman No. 1 filter paper was used with a solvent system containing 4:1:5 *n*-butanol, acetic acid, and water and a spraying agent made up of 0.5 g benzidine, 10 ml acetic acid, 10 ml 45% trichloracetic acid, and 100 ml 95% ethanol. A mixture of 10 ml filtrate and 25 ml 0.5% sodium polypectate was used from which samples were taken at 0, 12, and 24 hours. A 0.5% solution of monogalacturonic acid was used as the control. When the solution had risen to 1 in. of the top, the paper was removed, dried, and sprayed. It was then placed in an oven for 2 minutes at 90°C and examined. Monogalacturonic acid was indicated by a pink spot.

A test for the presence of cellulytic enzyme (Cx) in the extract of rotted potatoes was carried out by the viscosity reduction method using a 1.5% solution of methyl cellulose.

EXPERIMENTAL RESULTS.—*Maceration tests.*—The relation of pH to the macerating activity of a frozen extract of rotted sweet potatoes was determined with carrot root sections. The original extract had a pH of 4.4 and a maceration time of 25 minutes. When the extract was adjusted with 1*M* HCl and NaOH to pH 3.5, 4.0, 4.5, and 5.0, the respective maceration times were 41, 34, 30, and 32 minutes. Results were similar with 0.5*M* acetate buffer, but this buffer used at a ratio of 1:1 reduced maceration activity to about one fourth, even at the optimum pH, 4.5.

The fungus was grown at 20°C on wheat bran, sweet potato broth, Fernando's medium, and the last of these with 1% pectin. The filtrates were adjusted to pH 4.5 and tested for maceration activity with carrot root sections. The respective maceration times were 90, 106, 99, and 151 minutes. It was obvious that none of the artificial substrates was as effective as extract of rotted sweet potato tissue in producing macerating enzymes. In other experiments, maceration of carrot sections with fresh extract of potatoes rotted at 20°C was completed in 12 minutes. When the extract of rotted potato was precipitated with ethanol, macerating activity was markedly reduced.

Characterization of enzymes produced.—Before attempting to determine PME activity of the various filtrates, the optimum pH for its activity was determined with an extract from rotted sweet potatoes. Adjustments of pH were made by 0.5*M* acetate buffers. Three pH levels, 4.0, 4.5, and 5.0, were tried. The respective amounts of methoxyl groups removed by 1 ml of the extract in 3 hours at these pH levels were 2.23, 4.50, and 4.30 mg. PME activity of several filtrates was measured at pH 4.5. No PME activity was detected in filtrates of sweet potato broth, Fernando's medium, Fernando's medium with pectin, and a 2% solution of the crude enzyme preparation. In the filtrate of the wheat bran culture grown at 20°C there was slight PME activity, the amount of methoxyl groups removed by 1 ml in 3 hours being 0.20 mg. When the extract from sweet potatoes rotted at 20° was tested, the methoxyl groups removed by 1 ml of the sample was 6.2 mg. The healthy sweet potato extract removed 0.54 mg. It was evident that PME was produced abundantly when the fungus was growing on the host substrate, but little or none was formed in the culture media used.

Winstead and Walker (6), working with several plant pathogens, reported the highest PME activity in culture filtrates of *Pseudomonas solanacearum* E. F. Sm., the amount of methoxyl groups removed per ml in 3 hours being 3.014 mg. Still-higher PME activity was recorded by Echandi and Walker (1) in culture filtrates of *Sclerotinia sclerotiorum* (Lib.) d By., the methoxyl groups removed per ml in 3 hours being 4.09 mg. The PME activity of rotted sweet potato at 20° was higher than in either of the above-mentioned cases.

The pectin-splitting activity of extracts and filtrates was determined in 2 ways: 1) by measuring the rate of reduction in viscosity of 1.5% pectin solution; 2) by measuring the rate of release of reducing groups from a 0.5% solution of sodium polypectate. Using an extract of rotted potatoes and 0.5*M* acetate buffers, the pH optimum for the pectin-splitting activity was measured and found to be 4.5, by both the viscosity reduction and the reducing group tests. The rates of reduction in viscosity of 1.5% pectin solution and of release

of reducing groups from 0.5% solution of sodium polypectate by various filtrates and extracts were then determined at pH 4.5. The results are presented in Fig. 1.

The same filtrates and extracts tested for pectin-splitting enzymes were mixed with 0.5% sodium polypectate solution. After digestion for 24 hours the mixtures were tested for monogalacturonic acid by Ehrlich's basic lead acetate method. Results were positive in all cases but that of the extract of healthy sweet potato. The extract of rotted sweet potato and filtrates from cultures on wheat bran, sweet potato broth, and Fernando's medium containing 1% pectin were allowed to digest sodium polypectate, and the mixture was compared with 0.5% monogalacturonic acid and with a sodium polypectate blank by paper chromatography, immediately after mixing and at 12 and 24 hours. A positive test for monogalacturonic acid was secured immediately after mixing with rotted potato extract, wheat bran filtrate, and sweet potato broth filtrate. It appeared at 12 hours with filtrate of Fernando's medium containing pectin. No reaction was noted in the sodium polypectate blank at 24 hours.

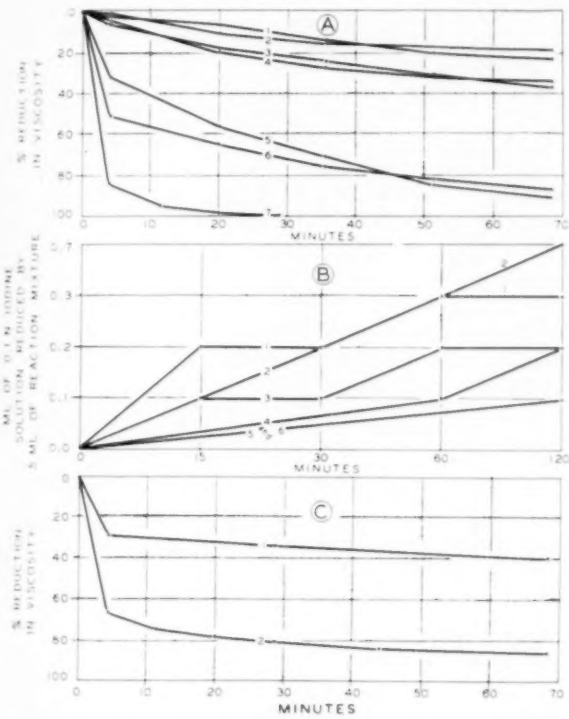

Fig. 1. A) Rate of reduction in viscosity of 1.5% pectin solution caused by 2) extract of uninoculated sweet potato tissue; 1, 3, 4) filtrates of cultures of *Rhizopus stolonifer* grown at 20°C on, respectively, Fernando's medium with pectin, Fernando's medium, and sweet potato broth; 5) a 2% solution of enzyme prepared from 7; 6) filtrate from culture grown on wheat bran at 20°; 7) extract of sweet potato tissue rotted by the fungus at 20°. **B)** Rate of hydrolysis of 0.5% sodium polypectate solution as measured by the release of reducing groups by: 1) extract of sweet potato rotted at 20°; 2) filtrate of wheat bran culture; 3) a 2% solution of enzyme prepared from 1; 4) filtrate of sweet potato broth culture; 5) filtrate of Fernando's medium culture; 6) filtrate of culture on Fernando's medium with pectin; all cultures were grown at 20°. **C)** Rate of reduction in viscosity of 1.5% solution of methyl cellulose by: 1) extract of rotted sweet potato; 2) a 2% solution of Pectinol D-100.

The rate of reduction in viscosity of pectin caused by the extract of rotted sweet potatoes was much faster than by any other filtrate. This could be attributed to the high PME content of the extract. However, the abrupt fall in viscosity during the first 4 minutes by the extract, wheat bran filtrate, and 2% solution of the enzyme strongly suggests that DP was present besides PG. In case of the filtrates from sweet potato broth, Fernando's medium, and Fernando's medium with pectin, the reduction in viscosity was very slow, indicating that the activity was due mostly to PG. Tests for PG in the extract of healthy potatoes were negative, and the slight reduction in the viscosity of pectin solution by this extract could have been due to PME.

A test for cellulase (Cx) in an extract of rotted sweet potatoes was made by the viscosity reduction test, using 1.5% solution of methyl cellulose. For comparison, the cellulase activity of a 2% solution of Pectinol D-100, a commercial pectinase preparation, was measured. The results in Fig. 1, show that a small amount of cellulase—enough to hydrolyze methyl cellulose—was present in the extract of rotted potatoes along with the pectolytic enzymes.

Discussion.—It was shown that *Rhizopus stolonifer* grown on culture media produced small amounts of PG and DP, and little or no PME. When the fungus was allowed to infect and grow on raw sweet potatoes, there was produced not only a greater quantity of pectin-splitting enzymes but also a large quantity of PME. The presence of cellulase (Cx) was also demonstrated in the extract of rotted potatoes. Harter and Weimer (2) reported that *R. nigricans* and *R. artocarpi*, both parasitic on sweet potatoes, secrete less pectinase when grown on sweet potato broth than do *R. chinensis* and *R. microsporus*, which are nonpathogenic on sweet potatoes. These results implied that production of extracellular pectinase by these fungi was not correlated with pathogenicity. If Harter and Weimer had evaluated the pectinase content of the extract of sweet potatoes rotted with *R. stolonifer* (*R. nigricans*), they probably would have reached a different conclusion.

Production of extracellular cellulase on cellulose-rich media by *Cladosporium cucumerinum* has been recently reported by Husain and Rich (4). It would be of interest to determine whether the cellulases produced by *R. stolonifer* and *C. cucumerinum* are of any significance in the maceration or distintegration of cell membranes of the respective host tissues.

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A DISEASE OF LADINO WHITE CLOVER CAUSED BY A YEAST, RHODOTORULA GLUTINIS VAR. RUBESCENS[1]

R. A. Kilpatrick[2]

SUMMARY

A previously undescribed fungus disease of Ladino white clover, caused by *Rhodotorula glutinis* var. *rubescens*, was found in New Hampshire. First leaf symptoms are chlorotic areas, which later coalesce, turn brown, and develop translucent water-soaked margins. Small light-tan pits develop on infected seeds. The fungus is readily isolated and grows well on potato dextrose agar. Growth is optimum near 24°C. Greenhouse inoculations suggest the fungus may become important only under prolonged high humidity. Seedlings of *Melilotus alba*, *Pisum sativum*, *Phaseolus vulgaris*, and 13 species of *Trifolium* were susceptible in greenhouse tests. Infected leaf tissues of *Trifolium maritimum* were readily stained with safranin. The fungus causes a breakdown of cellular structure.

During the past 2 growing seasons a leaf disease of Ladino white clover, *Trifolium repens* L., was observed in northern New Hampshire. In early June, 1957, symptoms were severe in about 25–40% of the leaves in plots at Colebrook, and the disease continued throughout the growing season. Seeds collected in September developed pits similar to those described by Lehman on soybeans.[3] Isolations from infected leaves and seeds always yielded a pink yeast identified as *Rhodotorula glutinis* (Fres.) Harrison var. *rubescens* (Saito) Lodder. Since no references to *Rhodotorula* spp. pathogenic to plants[4] could be found, various aspects of the disease and its causal agent were investigated.

Occurrence.—The disease has been found at 2 locations in New Hampshire, but it may also occur in other areas where humidity is high over long periods. In 1957, relative humidity reached 100% every night at Dover from May through September, except for 6 nights of 80% or above.

The disease was found on different species of *Trifolium* growing at the Strafford County Farm, Dover, and at Colebrook. The fungus was isolated from leaves of *Trifolium repens*, *T. medium* L., and *T. pannonicum* Jacq. Seeds of *T. repens*, *T. pratense* L., and *T. hybridum* L. showing symptoms yielded the fungus.

Symptoms.—On leaves of Ladino white clover, symptoms of the disease are similar to those of bacterial leafspot.[5] Infected areas are chlorotic and linear in early stages, and later coalesce, turn brown, and develop translucent water-soaked margins (Fig. 1-A). Lesions may cover 50–95% of the area of individual leaflets. Symptoms do not develop on petioles and stolons.

Pits in seeds vary in size and number (Fig. 1-B). Lesions are light tan and generally 0.5 mm in diameter, but may coalesce and cover 1 mm of the pericarp. Seeds are shriveled, immature, and generally nonviable. The fungus appears to be localized in the pericarp, but seeds occasionally germinate, becoming sources of primary infection.

Isolation.—The fungus was readily isolated with a dilution technique. Two localized lesions were minced in 10 cc of sterile distilled water containing 2 drops of 25% lactic acid. Two drops of a 1:10 or 1:100 dilution were placed on solidified Difco PDA (potato-dextrose agar) in Petri dishes and incubated 4–6 days at 18°C. Discrete colonies developed and were transferred to tubes or other dishes of PDA.

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[5] Jones, L. R., Maude M. Williamson, F. A. Wolf, and Lucia McCulloch. 1923. Bacterial leafspot of clovers. J. Agr. Research 25: 471-490.

Two other yeasts, *Cryptococcus albidus* (Saito) Skinner and *Rhodotorula mucilaginosa* (Jörgensen) Harrison, were also isolated, but attempts to infect plants with these species were unsuccessful.

CULTURAL STUDIES.—Growth studies were made on potato-dextrose, carrot root, carrot leaf, corn-meal, and water agar. *R. glutinis* var. *rubescens* was streaked in 2 places in 4 dishes of each medium and incubated 10 days at 18°C. Growth of the fungus was most abundant on PDA, good on carrot leaf and root agars, slight on corn-meal agar, and absent on water agar. Results were similar when the test was repeated.

Growth rates of *R. glutinis* var. *rubescens* on PDA were determined at 3.5, 11.5, 18, 24, and 30°C, maintained within ±1°C. Six Petri dishes, each containing a 5-mm agar disk of the fungus on 20–25 cc of PDA, were incubated 14 days at each temperature, and the test was repeated. The respective average radial growths in 14 days were 4, 6.5, 19, 26, and 19 mm. A third test, at 1° and 35°C, produced no growth. The cardinal temperatures are optimum near 24°C, maximum between 30° and 35°C, and minimum between 1° and 3.5°C. In all cases the fungus grew slowly when transferred as a disk.

INOCULATION.—Various inoculation techniques were tried in the greenhouse. Infection was heaviest from rubbing the fungus on the underside of a leaf with the fingers and incubating plants 72 hours in a moist chamber. For 4–5 days following the incubation period, plants were kept on a greenhouse bench during the day and incubated at night in a moist chamber. This simulated field conditions to some extent. Under these conditions, symptoms developed 7–10 days after inoculation. In contrast, plants removed to a dry atmosphere after the 72-hour incubation period developed symptoms very slowly.

HOSTS.—Approximately 6-week-old seedlings of various species of the following legumes were inoculated in the greenhouse with *R. glutinis* var. *rubescens*: *Trifolium ambiguum* Bieb., *T. angustifolium* L., *T. hybridum*, *T. incarnatum* L., *T. lappaceum* L., *T. lupinaster* L., *T. maritimum* Huds., *T. medium*, *T. pratense*, *T. repens*, *T. scutatum* Boiss., *T. tomentosum* L., *T. xerocephalum* Fenzl., *Melilotus alba* Desr., *Pisum sativum* L., and *Phaseolus vulgaris* L. When rated for susceptibility, 14 days after inoculation, all species proved susceptible. The amount of leaf infection varied with species, though symptoms were identical on all plants. The translucent water-soaked areas were more distinct in greenhouse-inoculated plants than in plants diseased in the field. *Trifolium angustifolium* and *T. maritimum* were the most susceptible species tested. Leaflets of *T. medium* developed a veinal necrosis, first appearing at the base of the leaflet and eventually spreading into the petiolule. Other species developed this symptom, but less distinctly.

HISTOLOGICAL STUDIES.—Pieces of diseased leaflets of *T. maritimum* were fixed in FAA (formalin–acetic-acid–alcohol)[6] to determine the histology of host cells. They were progressively dehydrated in alcohol, and infiltrated first with xylene and later with paraffin. Sections 8 μ thick were cut. Since no information was available on staining yeast cells in plant tissues, sections were differentially stained with fast green and safranin. Examination of the stained sections revealed the following: Upper and lower epidermal cells and the palisade cells were 1 layer thick, whereas the remainder of the leaf consisted of several layers of spongy parenchyma cells (Fig. 2-A). Cells in uninfected leaf tissue were regularly arranged, whereas cells in infected areas were disorganized. Safranin accumulated in cell walls of newly infected areas (dark areas, Fig. 2-BCD). As infection progressed,

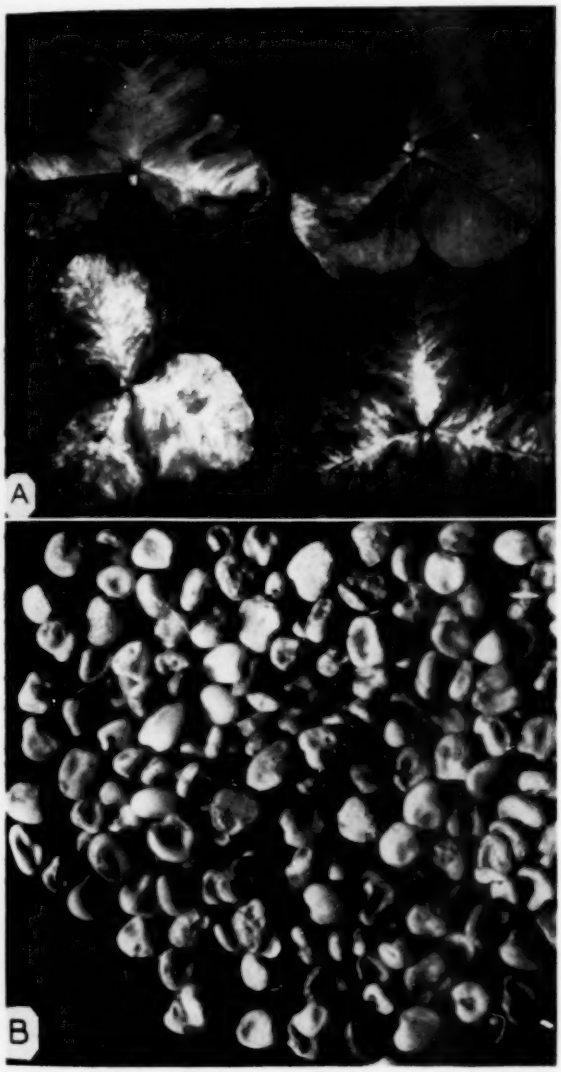

Fig. 1. Field symptoms of Ladino white clover incited by *Rhodotorula glutinis* var. *rubescens*. **A)** Leaves. No symptoms, upper right, compared with diseased leaves. **B)** Seeds. Note the pits within the pericarp.

[6] Johansen, D. A. 1940. Plant microtechnique. McGraw-Hill, New York, p. 41.

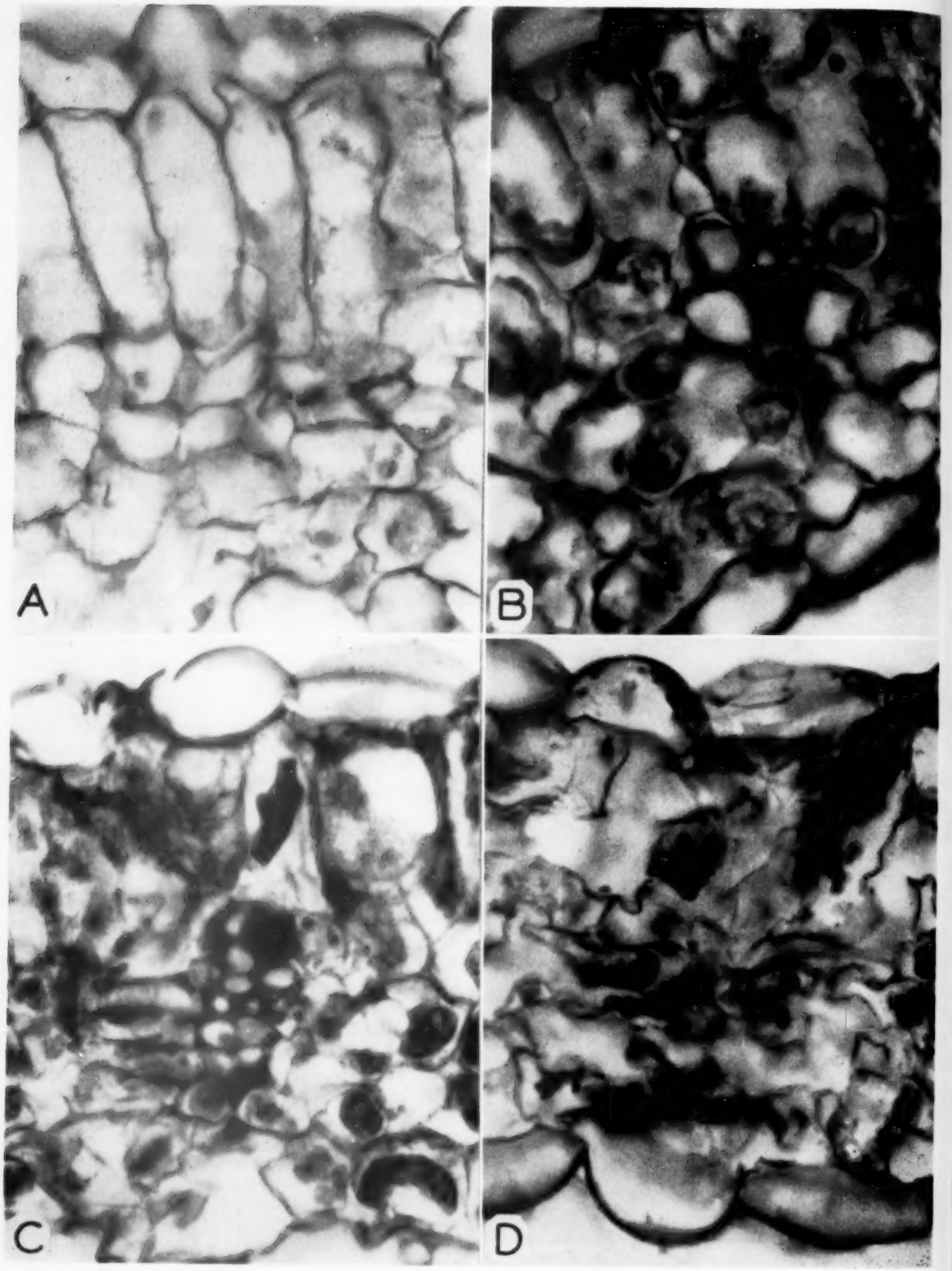

Fig. 2. Cross sections of leaflets of *Trifolium maritimum* infected by *Rhodotorula glutinis* var. *rubescens*. **A)** healthy tissue. **B, C, D)** cellular destruction showing relation to degree of infection. Note progressive breakdown. ×990.

the cell walls tended to break down and the arrangement became irregular (Fig. 2-D). Since the fungus has no mycelium, it is suggested that it produces a toxin that causes breakdown of cell walls and eventual premature defoliation.

OVERWINTERING.—Leaflets from a plant of *T. pannonicum* infected with *R. glutinis* var. *rubescens* in 1957 were collected on May 10, 1958. Leaflets were rinsed, placed in a Waring Blendor containing 150 cc of distilled water, minced 2 minutes, and incubated 18 hours at room temperature. Two drops of the suspension were then placed in 8 cc of sterile distilled water and agitated lightly, and 2 drops of the diluted suspension were transferred to each of 6 Petri dishes, each containing PDA and 2 drops of 25% lactic acid. After incubation for 6 days at 18°C, *R. glutinis* var. *rubescens* and *Cryptococcus albidus* colonies had developed in all dishes.

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DURHAM

SEROLOGICAL STUDIES OF VIRUSES INFECTING THE CEREAL CROPS. II. THE ANTIGENIC CHARACTERISTICS OF WHEAT STREAK MOSAIC VIRUS AS DETERMINED BY THE COMPLEMENT FIXATION TECHNIQUE[1]

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SUMMARY

Rabbits were successfully immunized with several different preparations of wheat streak mosaic virus (WSMV). Since this virus is present in relatively low concentration in the host plant tissue, it was purified with much difficulty, using differential centrifugation, density-gradient centrifugation, and zone electrophoresis. A toxic response in many rabbits injected with partially purified antigens or with highly purified antigens obtained from electrophoresis was reduced considerably by giving injections on alternate weeks. Three consecutive injections produced an immune serum of adequate titer. Since only very small amounts of virus were available for any one injection, all of the antigens were given in a mixture with a mineral oil adjuvant. A component of wheat remained in the antigens through several centrifugation procedures and electrophoresis. When healthy wheat plant sap was used as the test antigen, antibodies specific for this component could not be detected by the complement fixation technique, or were present in small amounts. When this sap was subjected to high-speed centrifugation or rate density-gradient centrifugation, the non-virus antibodies were detectable in significant amounts. Since the proportion of the wheat component to virus is high in purified antigen preparations, the presence of this component must be considered in evaluating data from cross-reaction experiments.

Wheat streak mosaic virus (WSMV), *Marmor virgatum* var. *typicum* McK., was first isolated in 1932, from diseased wheat found at Salina, Kansas (3). Almost every year since then great damage to winter wheat has been reported throughout the Great Plains Region of the United States and Canada. In 1952 Slykhuis (8) reported 70–100% infection and radical yield reduction in some fields in southern Alberta.

WSMV is transmitted by the mite *Aceria tulipae* Keifer (9). The virus is inactivated in plant sap at 55°C in 10 minutes, can be stored in desiccated tissue at room temperature for 34–40 days, and has an infectivity dilution endpoint of 5×10^{-3} (4). Because of the economic importance of this virus, an investigation of its antigenic properties was made. The results of this study and the use of the virus-specific serum for the identification and characterization of this virus are reported.

MATERIALS AND METHODS.—*Source of virus.*—The early investigations were carried out using yellow streak-mosaic virus (ATCC #29), obtained in 1952 from H. H. McKinney, Beltsville, Maryland.

In later experiments, newer cultures were obtained from the same source. These cultures were stored near 0°C under desiccation. Because of the possibility of introducing barley stripe mosaic virus into the WSMV stock through contaminated barley seed, frequent passage of WSMV was made through the vector. Fresh mite-transmitted cultures were always used when preparations were made for rabbit immunization.[3] These stock cultures of WSMV are identical to those described by Brakke (2).

Antigen preparations.—Since these studies were continued over a period of 2½ years, many different antigens were used to determine the best for immunizing and for serological test preparations. The early investigations were carried out with antigens prepared by differential centrifugation, whereas the later studies employed antigens purified through sucrose density-gradient columns.

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1) *WSMV antigens.*—For differentially centrifuged antigens, Michigan Amber wheat, grown on sand benches, was inoculated by the leaf-wiping method (5). Ten to 14 days later the plants were harvested, ground in an ordinary food chopper, and stored in ice cream cartons at −20°C. Antigenic material was obtained by expressing the sap from the thawed pulp through cheesecloth and subjecting the sap to 1 cycle of differential centrifugation, which consisted of 2 20-minute runs at 4000 G separated by 1 3-hour run at 78,000 G. The final pellet was taken up in a 0.05*M* neutral phosphate buffer-physiological saline (0.85% NaCl) solution. An additional cycle of centrifugation resulted in an extremely small yield and was used only once for intravenous injections into rabbits.

For the majority of antigen preparations, a combination of high-speed centrifugation and the sucrose density-gradient techniques was used in purification studies. Brakke (2) has reported in detail on the latter procedure. The five steps used to prepare the antigens are described below.

Step 1. Initial clarification and concentration.—Although Michigan Amber wheat was used most frequently, the Cheyenne variety was occasionally substituted. Only the youngest infected leaves of wheat were harvested. These were weighed and ground in a food chopper. At first, the resulting pulp was mixed in a Waring Blendor with 2 ml of 0.01*M* K_2HPO_4 per g of tissue. The sap was expressed through cheesecloth, heated 1½ hours at 40°C, clarified by low-speed centrifugation, and concentrated by high-speed centrifugation for 2½ hours at 78,000 G. Later, the procedure was altered by adding an equal volume of the phosphate solution to the pulp and by decreasing the time for heating the sap to 45 or 60 minutes. The virus in the pellet obtained from high-speed centrifugation was resuspended in 0.01*M* neutral potassium phosphate buffer. A Potter homogenizer was used to resuspend the virus.

Step 2. Rate preparation.—Gradient columns made by layering 4, 7, 7, and 4 ml of 100, 200, 300, and 400 g, respectively, of sucrose per liter were made on a previous day. Two to 4 ml of the virus preparation from Step 1 were floated on each column. These were centrifuged 2 hours at 5°C in the SW-25 rotor of the Spinco Model L centrifuge at 25,000 rpm. With a syringe and a J-shaped 4-in. hypodermic needle, a visible zone was removed from a depth of about 2.5–3.5 cm.

Step 3. Equilibrium preparation.—In early experiments, tubes containing layers of 1.2 ml each of 500 and 700 g of sucrose per liter were made on a previous day. These small plastic tubes, 0.5×2.0 in., were then filled by floating the preparation from Step 2 on the sucrose solution. They were centrifuged 4 hours at 39,000 rpm in the SW-39 rotor. In later experiments the gradient columns used were made by layering 4 ml each of 300, 400, 500, and 600 g of sucrose per liter. Centrifugation was carried out for 7 hours in the SW-25 rotor at 25,000 rpm. In each case, the zone immediately below the green band was removed with a syringe and bent needle.

Step 4. Zonal electrophoresis.—U-tubes containing a density gradient (1) were prepared on a previous day using solutions of 300, 400, 500, and 600 g of sucrose per liter in a buffer solution of 0.01*M* KCl, 0.005*M* K_2HPO_4, and 0.0005*M* KH_2PO_4, pH 7.8. The virus preparation from Step 3 was mixed with buffer so that it would float at about a depth of 1.5 cm. in one arm of the U-tube. A current of 350 volts and 26 ma. per 6 U-tubes (which dropped to 10 ma. during the run) was used for an overnight run of 15 hours in the electrophoresis apparatus designed by Brakke. The visible zone was removed with a syringe and bent needle.

Step 5. Final concentration.—Following any of the purification procedures involving a sucrose solution, the virus was separated from the sucrose by dilution and centrifugation for 2 hours at 39,000 rpm in the SW-39 rotor of the Spinco Model L centrifuge. The resulting pellet was suspended in 0.05*M* neutral phosphate buffered saline.

The antigen most frequently used was the rate preparation obtained from Step 2. This preparation included Steps 1, 2, and 5 of the preceding description. An equilibrium preparation of antigen included Steps 1, 2, 3, and 5, which means that an equilibrium antigen was always preceded by the rate separation technique. The virus preparations subjected to electrophoresis employed all 5 steps of the above purification procedure. Since much time was involved in the purification procedures, several experiments were conducted to measure the stability of the antigens. Virus solutions were tested serologically 1 week after their preparation, and only a twofold drop in the antigenic unit was observed.

2) *Healthy wheat antigens.*—Michigan Amber wheat plants of the same age as those infected with WSMV were harvested and ground in a food chopper, and the pulp stored at −20°C. For immunization and for serological tests, sap from the pulp was expressed through cheesecloth and clarified by centrifuging 30 minutes at 4000 G.

In later experiments, the youngest leaves from healthy Cheyenne wheat were picked and ground in a food chopper. The sap was expressed through cheesecloth and treated in a manner similar to WSMV antigen in that it was heated 45–60 minutes at 40°C and centrifuged in the #30 rotor of the Spinco Model L centrifuge for 30 minutes at 20,000 rpm and 2½ hours at 30,000 rpm. In some experiments, the concentrated sap was further purified by rate and equilibrium density-gradient centrifugation. In every case, the material remaining in the rate or equilibrium zones at the same depth as WSMV was removed, diluted with buffer, and thrown out of solution by high-speed centrifugation as described in Step 5 above.

Antiserum preparations.—The majority of injec-

tions of virus material were given in a mixture with a mineral oil adjuvant (7). A suspension containing a ratio of 10:9:1 of virus, bayol F, and Arlacel A, respectively, was emulsified in a rubber-capped serum bottle using a syringe and short 18-gauge needle. At any one time, a total volume of 5 or 10 ml was made, and this emulsion was injected into the calf or thigh muscle of the hind leg of a rabbit. Depending on the final yield of virus protein in an antigen preparation, one or both legs were used; no more than 2.0 ml of adjuvant mixture was injected into one leg at any one time.

One rabbit (Number 19) was held on test with WSMV antigen for the entire investigation period of 2½ years. This rabbit received antigen purified by differential centrifugation in the primary immunization period and in the first booster injection series, given in the 15th and 17th week after the initial injection. A second booster injection series, given in the 50th, 51st, 52nd, and 53rd weeks, employed highly purified WSMV that had been taken through all of the purification steps described previously, including electrophoresis. A similar injection of highly purified antigen was given as the fourth booster dose, in the 98th week. The third booster and fifth booster, given in the 80th and 124th weeks, were prepared by purifying the virus through the rate step and absorbing the plant contaminants with antisera specific for healthy wheat and for partially purified wheat antigens.

Other rabbits were injected with virus prepared by differential centrifugation (Number 20) only, by the electrophoresis procedure (Number 13) only, by the rate centrifugation and absorption with sera specific for the wheat plant (Numbers 129 and 130) only, or by combinations of the above methods of preparation (Numbers 32, 114, and 116). The data compiled from the primary phase of immunization of eight rabbits are summarized in Table 1, Part A. The data representing the results from giving 2 rabbits additional booster doses of antigen are summarized in Table 1, Part B. The rabbits were usually inoculated in pairs, and, as indicated in Part B, rabbit 19 was paired with rabbit 32 during part of the booster series of injections. The serum of rabbit 13 showed less antibody after a booster series, presumably because the virus content of the antigen preparation was not adequate to give a better response. A booster series of injections given to rabbits 20, 114, and 116 increased by twofold the maximum antiserum dilution

TABLE 1.—*A summary of the procedure used to immunize 8 rabbits with WSMV. A study of the influence of the injection technique and purification of the antigen on the antibody content of the immune serum*

Part A. Measurement of serum taken during the primary phase of immunization

	Rabbit number				
	19 20	32	13	114 116	129 130
Number of injections in series	2	1	3	4	3
Interval between injections (days)	7		7	14	14
Purification of antigen[a]	d.c.	d.c. abs	1–5	1–5	1–2+5 abs
Volume of antigen given (ml)[b]	3.0 0.4*	4.0	5.0	7.6	5.5
Maximum serum titer,[c] measured with:					
1. purified WSMV	128	128	256	128	256
2. clarified wheat sap	16	8	<4	<4	<4

Part B. Measurement of serum taken during the booster phase of immunization

	Rabbit number								
	19					32			
	1st	2nd	3rd	4th	5th	1st	2nd	3rd	4th
Time of booster series (weeks)[d]	15	50	80	98	124	33	63	81	107
Number of injections in series	2	4	1	1	3	4	1	1	3
Interval between injections (days)	14	7			14	7			14
Purification of antigen[a]	d.c.	1–5	1–2+5 abs	1–5	1–2+5 abs	1–5	1–2+5 abs	1–5	1–2+5 abs
Volume of antigen given (ml)[b]	4.1 2.4*	5.7	2.0	1.0	2.8	5.7	2.0	1.0	2.8
Maximum serum titer,[c] measured with:									
1. purified WSMV	128	128	512	128	512	256	64	64	128
2. clarified wheat sap	6	16	16	8	<4	4	<4	4	<4

[a] Purification of antigen: d.c. = differential centrifugation; 1–5 = purification through a preliminary high speed concentration (Step 1), through rate (Step 2) and equilibrium (Step 3) density gradient centrifugation, electrophoresis (Step 4), and through a high-speed centrifugation (Step 5); 1–2+5 = same as former except equilibrium centrifugation (Step 3) and electrophoresis (Step 4) are omitted; abs = prior to injection, antigen was absorbed with serum specific for healthy wheat sap (see Materials and Methods).

[b] Volume of antigen given: figures refer to total volume injected during series. All injections were given intramuscularly in a mineral oil adjuvant except (*), which was given intravenously in saline.

[c] Maximum serum titer: figures refer to reciprocal of antiserum dilution endpoint.

[d] Time of booster series: figures refer to the week after the initial injection during which the booster series began.

endpoint obtained in the primary phase. Rabbits 129 and 130 have not yet received booster doses of antigen.

A toxic effect from the WSMV antigen was observed several times throughout the study. In 2 cases, one of a pair of rabbits died while the remaining rabbit refused food and showed other evidences of a toxic response. In 4 cases, large cysts developed at the sites of injection and the rabbits refused food and showed signs of a similar toxic response for a time during the injection series. To overcome this, the time between inoculations was increased to 14 days during the final immunization injection series.

One rabbit (Number 6) was used for the preparation of antiserum specific for healthy wheat plant sap. This animal received a total of 15 ml of clarified sap injected intraperitoneally in doses of 2, 3, 5, and 5 ml over a period of 4 weeks. As with all other animals under study, periodic bleedings were made every other week for at least 12 weeks to trace the antibody response. In the 12th week following the initial injection, a booster dose of antigen mixed with a mineral oil adjuvant was given in the thigh muscle, and periodic bleedings were made following this injection.

Another rabbit (Number 122) was immunized with healthy wheat plant sap purified by high-speed concentration followed by rate density-gradient centrifugation. These antigen preparations were mixed with a mineral oil adjuvant and injected during 3 consecutive weeks. Periodic bleedings were made to trace the antibody response.

All serum samples were collected by cardiac punctures. Approximately 10 ml of blood were withdrawn. The blood was allowed to clot at room temperature, the clot was freed from the tube, and the blood was heated 30–45 minutes at 37°C. After incubation overnight at 4°C the serum was separated by centrifugation at 4000 rpm in a Clinical Model International centrifuge. The serum was stored at −20°C in sealed Wasserman tubes until used in serological tests. When the tests were completed, sera with the highest titer were pooled and stored more permanently, in rubber-capped serum vials or in sealed glass ampoules.

Serological test procedure.—The modified complement fixation test found to be adequate for brome mosaic and barley stripe mosaic viruses (6) was used in this investigation. The commercial source of complement and hemolysin,[4] and of sheep red blood cells[5] was the same as described for the other cereal viruses. All the test results were repeated at least twice before the data were interpreted. Greater than a twofold change in the dilution endpoint was necessary for differences to be significant.

Experimental results.—Rabbits immunized with WSMV prepared by differential centrifugation produced antisera whose maximum titer was relatively good, e.g., 1:128 (rabbits 19, 20, and 32; Table 1, Part A). As anticipated with this type of antigen, large amounts of antibody specific for the wheat plant were present in these sera. In the case of rabbit 19, the influence of the non-virus portion of the original antigen was observed after 50 weeks, even though the booster injections were made with highly purified antigen. Before injection, absorption of the differentially centrifuged antigens with antiserum specific for healthy wheat reduced the amount of non-virus antibody produced by rabbit 32 (Part A). Likewise, an eightfold reduction in non-virus antibody was observed from rabbit 20, which had received absorbed antigen during a second booster in the 39th week.

Rabbits immunized with WSMV that had been purified by rate density-gradient centrifugation (Steps 1, 2, and 5) or by the highly purified preparations from electrophoresis (Steps 1 through 5) produced maximum titers as good as from the above sera, or better. Moreover, serological tests with these sera and with healthy wheat plant sap antigen showed little or no cross-reacting antibody.

The optimum ratio of each antigen-antibody system was determined from grid titrations in which each twofold dilution of antigen was reacted with each twofold dilution of serum. The optimum ratio was defined as the highest dilution of antigen giving complete fixation with the least amount of serum (6). The amount of antigen in this ratio is defined as the antigenic unit. Considerable variation was observed in the antigenic unit of successive antigen preparations, even though laboratory conditions were standardized with regard to heat treatment of sap, centrifugation, and resuspension of the final pellets. For each preparation, the infected tissue was weighed and adjustments made so that the following concentrations were obtained from each step: 30–40 times initial concentration in Step 1, 10 times in the rate and in the equilibrium gradient centrifugation in Steps 2 and 3, and 4 times in the electrophoresis in Step 4. With a standard serum sample in 5 separate experiments, the antigenic unit of WSMV from equilibrium gradient centrifugation was as low as 1:64 and as high as 1:512. Equally variable antigenic units were obtained with high-speed and rate gradient preparations. The variation in total virus content of the antigen preparations was most probably the result of light and temperature changes in the greenhouse before and after inoculation.

For the serological tests, an attempt was made to use partially purified antigens that were absorbed with various amounts of antiserum specific for healthy wheat sap. These antigens were purified by rate density-gradient centrifugation, and, in 4 separate experiments, virus-serum mixtures of 1:1, 2:1, 4:1, and 9:1 were incubated overnight at 25°C, and centrifuged at low speed to remove the precipitate. In all cases the antigenic unit was reduced two- to fourfold below that of antigen from the same batch of WSMV to which normal rabbit serum or saline had been added. In 4 separate experiments the antigenic unit varied from 1:64 to 1:256, as in many other tests described in the previous paragraph. Anticomple-

[4] Merck, Sharp and Dohme, Philadelphia, Pennsylvania.
[5] Brown Laboratories, Topeka, Kansas.

mentary activity of the absorbed antigens was very high, being a fourfold dilution less than the unabsorbed antigen-antibody reaction. This made it difficult to interpret the significance of the results. Furthermore, the cross-reaction of these antigens with serum specific for healthy wheat was not markedly reduced. Since only a small margin of dilutions showed any significant results, this method of purification was abandoned as impractical for the complement fixation test.

In 3 experiments, antigen samples from both WSMV-infected and healthy wheat were withdrawn during each step in the purification procedure in order to measure the change taking place in total antigen content. These samples were reacted against a given serum sample, and the data are summarized in Table 2. Needless to say, the amount of antigen withdrawn at any one step had to be very small. Therefore, the number of dilutions in grid titrations for each antigen sample was limited. The cross-reactions of each of the WSMV and healthy wheat antigen samples with both virus and wheat immune serum are presented. The electrophoresis antigen gave good results with serum from rabbits 13 and 130. Conclusive evidence was not obtained with rabbit 19 serum.

Further studies were made on the cross-reaction of WSMV antigens with a number of antisera prepared in this laboratory. Tobacco mosaic virus and southern bean mosaic virus were purified by at least 3 cycles of differential centrifugation before being used for immunization. Barley stripe mosaic virus and the brome mosaic virus were purified by several cycles of differential centrifugation, and the properties of the antigens and technique used to immunize rabbits have been reported (7). Serological data from this laboratory have not yet been reported on latent potato X virus, tobacco ringspot virus, and elm ringspot virus. However, it is sufficient to report here that rabbits immunized with these three viruses received relatively impure preparations, e.g., the antigens were prepared by one cycle of centrifugation and remained quite green when injected into the animals. In the serological tests, WSMV antigens from high speed, from rate, and from equilibrium preparations gave no reaction with antisera specific for tobacco mosaic, southern bean mosaic, barley stripe mosaic, and brome mosaic viruses. These antigens did react at dilutions of 1:32 or less with antisera specific for latent potato X, tobacco ringspot, and elm ringspot viruses as well as with antisera of healthy wheat and tobacco plants. However, clarified sap antigens from healthy wheat plants cross-reacted with the latter group of virus antisera and with tobacco plant antiserum. The homologous healthy plant antigen-antibody reactions were, of course, greater than the heterologous reactions, but it was rather surprising to observe the relatively high percentage of common antigens among these plants.

Discussion.—This investigation has shown that WSMV is antigenic and, with certain modifications in technique, will stimulate antibody production in a rabbit to the same extent as barley stripe mosaic virus. The antibody response with this virus is not as good as when brome mosaic virus serves as the antigen. This, of course, has to do with the nature of the respective viruses, with their concentration in host plant tissue, and with the ease by which the virus can be separated from the host plant proteins. The overall immunological response in a rabbit to injections of WSMV resembled that of barley stripe mosaic virus, both in the maximum antibody titer, which

Table 2.—*Reciprocal of the antigenic unit (AU) and the antiserum dilution endpoint (ADE) from 3 experiments in which successive purification treatments of WSMV and wheat antigens were reacted with the same serum sample in complement fixation tests*

Antigens and purification treatment	#13-WSMV[a]		#6-Wheat[b]		#19-WSMV[c]		#6-Wheat[b]		#130-WSMV[d]		#6-Wheat[b]		#122-Rate[b]	
	AU	ADE	AU	ADE	AU	ADE	AU	ADE	AU	ADE	AU	ADE	AU	ADE
1. Clarified sap														
WSMV	8	32	512	128	32	256	512	128						
Wheat	<8	<8	256	64	64	16	256	128	16	8	512	128	16	8
2. Concentration (1)[e]														
WSMV	32	128	256	128	128	128	512	64	128	256	256	128		
Wheat	16	16	256	128	16	64	512	64	64	64			64	64
3. Rate (2)														
WSMV	64	128	32	128	32	128	32	64						
Wheat	16	8	16	128	<8	<8	<8	<8	16	64			8	64
4. Equilibrium (3)														
WSMV	64	256	32	128	64	128	<8	<8						
Wheat	<8	<4	<8	<4	<8	<8	<8	<8	8	4			<8	<8
5. Electrophoresis (4)														
WSMV	64	256	16	64	<8	<64	<8	<8	64	256	16	32		

[a] Rabbit immunized with WSMV from electrophoresis. Sample, 7th week.
[b] Rabbits immunized with clarified wheat plant sap (#6) and with wheat sap purified by rate centrifugation (#122).
[c] Rabbit immunized with WSMV from differential centrifugation (primary series and 1st booster), from electrophoresis (2nd booster), and from rate centrifugation with absorption before injection (3rd booster). Sample, 5th week of 3rd booster series.
[d] Rabbit immunized with WSMV from rate preparation absorbed prior to injection. Sample, 6th week.
[e] Numbers in parentheses refer to the purification steps described in Table 1 and in Materials and Methods.

ranged from 1:128 to 1:512 in the primary and in the booster injection series, and in the short period, in which the antibody titer remained at a maximum. This maximum peak remained for only 2–4 weeks. As mentioned in the previous article (6), this is in contrast to tobacco mosaic virus or southern bean mosaic virus, in which the antiserum cointained the maximum amount of antibody for 30–40 weeks after injection of the virus in an adjuvant. One major difference between WSMV and barley stripe mosaic virus was that a single injection of barley virus in adjuvant was sufficient to achieve the same result as at least 3 injections of WSMV.

Mention has been made of the fact that successive batches of WSMV varied considerably in virus content. The variation was seen in infectivity dilution endpoints as well as in the antigenic unit of the serological tests. In any one preparation of a purified immunizing antigen no attempt was made to measure the actual weight of virus, since the final yield of virus was extremely small. However, the total of purified virus probably never exceeded 1 mg. This, in an adjuvant, was injected into 2 or 4 rabbits. In view of the total amount of virus protein given to any one rabbit, the antibody response was surprisingly good. Aside from affecting the amount of virus in the antigen used for immunization, the variation in virus content of antigen preparations made it extremely difficult to compare data from the serological tests in a conventional manner. Usually, the antigenic unit is determined from a grid titration of antigen and serum, the weight of virus in the antigenic unit is calculated, and this weight is used as a constant in determining the antibody content of all homologous and heterologous sera. The antigenic unit can be estimated with good accuracy when new antigens are prepared by using a serum previously standardized to a weighed virus preparation (10). In the present studies, the weight of virus in the antigenic unit of any one antigen could not be estimated, because of small yields from rate, equilibrium, or electrophoresis preparations, or because of the presence of large amounts of plant antigens in clarified sap and in the initial concentration procedure. In Table 1, the antiserum dilution endpoints were measured by using a single optimum dilution of antigen determined by the standard procedure. In Table 2, grid titrations were made so that any shift would be observed in the optimum reacting proportions of antigen and antibody. Such a shift was observed. The greatest change in optimum ratios should take place between measurements of clarified sap and of initially concentrated antigens. Since rate and equilibrium treatments are essentially purification, not concentration, techniques, there should not be a significant change in optimum ratios between these antigens and a given serum unless there were experimental error in removing the respective zones from the sucrose density-gradient. Where no virus was present, and consequently no zone marker, the removal of all the wheat component equal to that associated with the virus was practically impossible. This may explain the rapid shift of optimum ratios of wheat antigens from rate and from equilibrium preparations, as seen with all sera in Table 2.

From the results obtained in this study, two WSMV antigen preparations produced satisfactory antisera. These antigens were purified 1) by rate and equilibrium density-gradient centrifugation and electrophoresis, and 2) by rate centrifugation followed by absorption with sera specific for healthy sap and for a purified wheat fraction. From the data of Table 2 it is evident that the electrophoresis antigen produced the most specific antiserum. Rabbit 13 antiserum gave little cross-reaction to clarified wheat sap antigen, and, although this cross-reaction increased when the wheat antigens were purified and concentrated, the antiserum dilution endpoint with virus antigen remained 8- to 32-fold greater than the reaction with the non-virus antigen. The serum of rabbit 130, immunized by injecting WSMV absorbed with healthy wheat antiserum, contained some non-virus antibody, more than that from rabbit 13 but less than that from rabbit 19. This effect was probably due to the state of the antigen used for the injections. Even though the precipitate of aggregated wheat antigen and antibody was removed by centrifugation, the clear supernatant fluid probably contained a large amount of the unaggregated soluble complexes of this antigen-antibody system as well as the virus, both of these being antigenic. Since the absorbed antigen preparation required the least number of steps in purification, it thereby left more virus available than did the electrophoresis procedure. This method was adopted for preparing serum to be used in routine identification tests such as chloroplast agglutination and precipitin techniques.

Table 2 demonstrates the presence in all WSMV sera of a relatively high proportion of antibody specific for a wheat fraction. In dealing with viruses that are relatively stable but in low concentration in the host plant tissue, it is important to consider the fraction of plant protein that remains in solution with the purified virus. This study has demonstrated conclusively that a very antigenic wheat plant component cannot be separated from the virus even after electrophoresis. Apparently, the proportion of this component to the virus was very high in purified preparations. The preparation of a serum specific for the complete wheat plant is not a sufficient control for this study. Included as a control must be a serum specific for the fraction of the wheat plant that is at the same depth as the virus in density gradient columns.

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HOT-WATER TREATMENT OF LAMBERT CHERRY BUDSTICKS INFECTED WITH NECROTIC RUSTY MOTTLE VIRUS[1]

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SUMMARY

Budsticks from sweet cherry (variety Lambert) infected with necrotic rusty mottle virus were treated in a hot water bath at 50°C for 10, 13, and 15 minutes, and at 52°C for 5, 8, and 10 minutes. Virus inactivation occurred in all treatments. Seven of 10 Lambert trees that received buds treated at 50°C for 15 minutes and at 52°C for 10 minutes survived at least 2 years. Five of the 7 were still symptomless at that time. Inoculation bud shields were alive in 3 of the 5 trees. Treatments at 50°C for 10 and 13 minutes and at 52°C for 5 minutes were about 50% effective in inactivating the virus.

Thermal inactivation "in vivo" has been accomplished with several stone fruit viruses (1, 2, 3, 4). It was thought that this method might be used to separate certain viruses from a mixture when other methods of separation could not be applied. During the course of such investigations, Lambert cherry infected with necrotic rusty mottle virus was subjected to various treatments in a hot water bath. This paper deals with that part of the larger investigation.

The culture used contained stone fruit ringspot virus along with necrotic rusty mottle virus. Recent bud inoculations with this mixed culture resulted in 100% transmission of both viruses to Lambert trees. Past failures to obtain a similar high rate of infection with some cultures seemed to be associated with early death of inoculation buds, presumably from ringspot shock reaction. If the inoculation tissue lived at least 1 month, necrotic rusty mottle was transmitted. During 9 years of investigating different phases of this disease, transmission has been consistent and uniformly high.

Methods.—Current-season budsticks were selected from Lambert trees showing severe symptoms of necrotic rusty mottle. Bark on yearling and older wood showed typical discrete blisters and more general necrosis. Leaf symptoms when the experiment was initiated were mild, but present on all sticks selected. Five sticks, each containing 5 buds, were used for each treatment. Budsticks were immersed in a hot-water bath at 50°C for 10, 13, and 15 minutes, and at 52°C for 5, 8, and 10 minutes. Temperature was controlled to within 0.1°C. A cold-water post-treatment dip reduced stick temperatures rapidly. To check bud survival, 4 buds from each stick were immediately inserted in a line in convenient-sized branches of vigorously growing trees of the sweeet cherry variety Van. To test virus inactivation, the remaining bud from each stick was inserted singly in Lambert trees growing in a nursery row. These trees were later lifted in the usual way and replanted at a spacing of 12×6 ft.

Results.—Survival of treated buds or bark shields was essential to determine virus inactivation, since some tissue union is necessary to obtain transmission. Survival was excellent after treatments of 50°C for 10 or 13 minutes and 52°C for 5 minutes: 59 of 60 buds or shields put in Van were alive after 6 weeks. Of the 14 live Lambert test trees in this group, 7 had not developed symptoms 2 years after treatment. In 4 of these 7 trees, there was live tissue of the inoculation buds or shields 8 months after treatment. Thirteen of 15 inoculation buds placed in Lambert trees survived at least 6 weeks after treatment. Data for all treatments are shown in Table 1.

Survival was considerably lower in buds and shields treated at 50°C for 15 minutes and 52°C for 8 and 10 minutes. None heated at 52°C for 8 minutes survived even 6 weeks in Lambert, although about half survived at least 1 year in Van shoots. Eleven of 15 Lambert trees each inoculated with a treated bud survived at least 2 years. Eight of these trees had not shown symptoms of necrotic rusty mottle during

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TABLE 1.—*Effect of hot-water treatment on bud or shield survival and on transmission of necrotic rusty mottle virus from infected sweet cherry*

Treatment		Bud or shield survival in Van (20 used)		Lambert trees single-bud inoculated				
							Without symptoms	
°C	Minutes	6 weeks	32 weeks	No. used	With buds or shields alive at 6 weeks	With symptoms	Shield alive[b]	Shield dead
50	10	19	19	5	5	3	1	1
	13	19[a]	20	5	3	1	2	1
	15	9	7	5	5	1	1	2
52	5	20	20	5	5	3	1	1
	8	8[a]	9	5	0	1	0[c]	3
	10	5[a]	6	5	2	1	2	0
Untreated		20	20	5	5	5		
Totals				35	25	15	7	8

[a] The 32-week reading was considered more accurate than the 6-week reading, since bud and shield tissue was scraped and exposed.
[b] Readings taken March 19, 1957, about 8 months after treatments were made.
[c] Buds and shields were dead within 6 weeks after insertion into healthy Lambert.

this time. The inoculation bud shields were alive at least 8 months after budding in 3 of the 8 negative trees. If one excludes the data from Lambert inoculations with buds treated at 52°C for 8 minutes, the remaining data carry considerably more significance.

Seven Lambert trees lived out of 10 inoculated with buds treated at 50°C for 15 minutes and at 52°C for 10 minutes, and 5 of them were symptomless after 2 years. Seven of these 10 treated buds survived at least 6 weeks after budding, and 3 survived at least 8 months. It is not known if the Lambert trees that died during the first year after inoculation were infected with necrotic rusty mottle, since they died soon after leafing out. Considering the number of trees alive after 2 years, and the usual 95–100% transmission from Lambert tissue affected with necrotic rusty mottle, even when mixed with ringspot, 50°C for 15 minutes and 52°C for 10 minutes seem to have been about 70% effective in eliminating necrotic rusty mottle virus from infected propagating tissue. Treatments at 50°C for 10 and 13 minutes and 52°C for 5 minutes were 50% effective.

Symptoms of ringspot shock were severe on all inoculated trees and 5 trees did not survive. These were not included in the data presented in Table 1. The treatments themselves may in some way have made the shock symptoms more severe, since all check trees inoculated with untreated buds survived, as did all trees inoculated with buds treated for the minimum time at each temperature.

DISCUSSION.—If buds containing little or no virus when treated survived better than buds containing enough virus to transmit, erroneous conclusions could be drawn. Apparent virus inactivation might simply be differential survival of treated tissue.

The results obtained from these treatments furnish evidence that necrotic rusty mottle virus was actually inactivated in the treated tissue. Previous experience with this virus, and the high incidence of transmission, indicate that it is essentially completely systemic in current-season budsticks of Lambert trees showing advanced symptoms of disease. Failure to obtain transmission after hot-water treatment, especially when treated tissue remained alive in test trees for more than 6 weeks, strongly indicated virus inactivation.

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PHYTOPATHOLOGICAL NOTES

Evidence of Seed Transmission of Angular Leaf Spot of Bean. S. H. Orozco-Sarria, and C. Cardona-Alvarez.[1] Angular leaf spot of bean (*Phaseolus vulgaris* L.), caused by *Isariopsis griseola* Sacc., has long been known as a foliage disease. Cardona-Alvarez and Walker,[2] using various lots of seed from diseased plants, concluded that the fungus was not carried in or on the seed. There are no other reports on this aspect of the disease. A recent chance observation in Colombia indicated that *I. griseola* was possibly transmitted on the seed.

In all tests the seed was surface-sterilized by immersing it briefly in alcohol, soaking it 3–5 minutes in mercuric chloride (1:1000), and finally washing it 5 times in sterile distilled water.

Seed about 1 month old from breeding line 143 was separated into 2 groups: 1) apparently healthy seed, and 2) seed showing reddish-brown discoloration of the coat. After being surface-sterilized, subgroups of 5 seeds from each lot were transferred to, respectively, PDA (potato-dextrose agar) plates, water-agar plates, or Petri dishes containing moist filter paper, and incubated at 24°C. By the 3rd day some of the discolored seed showed a light- to dark-gray fungal growth on the hilum. At 5 days, 50% of the discolored seed in all 3 subgroups showed the fungal growth, whereas none was evident on the healthy seeds. Similar tests with several other lines and varieties gave negative results. The fungus on the seeds of line 143 was identified as *I. griseola*. Its pathogenicity was proved by inoculating several bean varieties.

Discolored seed of line 143 was stored at room temperature (22°C) and sampled every 3 months for presence of the fungus. The seed was surface-sterilized after storage and transferred to PDA or placed directly in Petri dishes containing moist filter paper. The percentage of survival of the fungus in the seed declined in storage, from 50% at 1 month to 10% at 9 months. No survival was observed at 12 months.

To evaluate the importance of seed infection in disease development in the field, 3 4-month-old seed lots of line 143 were used: 1) surface-sterilized discolored seed, 2) healthy seed sprayed with a spore suspension of *I. griseola* and allowed to dry, and 3) surface-sterilized healthy seed. The seed was planted in sterilized soil in sterilized flats in individual moist chambers at 20–24°C, and watered daily. The first symptoms of angular leaf spot were observed 13 days after planting: typical disease lesions were noted on the cotyledonary leaves in lots 1 and 2, but no symptoms were seen in lot 3. These observations clearly show that fungus in or on the seed could be a source of primary infection. Infection of the cotyledonary leaves was also obtained on plants from surface-sterilized discolored seed of line 143 that had been stored for 6 and 9 months.

Growth of the fungus on surface-sterilized seed in agar proves that the pathogen is borne in or under the seed coat or the hilum. The disease lesions noted on the cotyledonary leaves of seedlings from surface-sterilized healthy seed that was sprayed with a spore suspension of *I. griseola* indicate that spores on the seed coat may be a source of primary inoculum. No reason is apparent why the pathogen is seed-transmitted by line 143 but not by other lines or varieties.

Survival of the fungus for 9 months on plant debris or seed left in the field after harvest is probably more important than survival in stored seed as a source of primary inoculum in Colombia (tropical or similar regions). Colombian soils, unlike soils in other parts of the world, are not subjected to extremes of heat and cold between bean crops. Therefore, in areas where there is limited survival in the soil, transmission of *I. griseola* through stored seed may be relatively important.—"Tulio Ospina" Agricultural Experiment Station, Medellín, Colombia.

A Major Gene Locus for Compatibility in Trichometasphaeria turcica.[1] R. R. Nelson.[2] Northern leaf blight of corn, caused by *Helminthosporium turcicum* Pass., occurs in many of the world's major corn-growing areas. In certain years the disease is destructive in the Corn Belt and the Southeastern states. Inheritance of resistance, studied extensively, is controlled by many genes and inherited as a quantitative character.[3] It has been recognized that a more effective breeding program could be achieved if the genetic behavior and variability of the pathogen were known. The cultural and pathogenic variability of the conidial stage has been investigated.[4] The ascigerous stage of the fungus was recently described[5] as *Trichometasphaeria turcica* (Pass.) Luttrell, thus making possible basic investigations on the genetic behavior of the pathogen. Luttrell[5] obtained mature perithecia of *T. turcica* by pairing compatible conidial isolates, and concluded that the fungus is heterothallic. Production of perithecia was usually sparse and inconsistent.

[1] Agronomist and Director, respectively, Bean Improvement Program, Colombian Ministry of Agriculture, Medellín, Colombia.

[2] Cardona-Alvarez, C., and J. C. Walker. 1956. Angular leaf spot of bean. Phytopathology 46: 610-615.

[1] Cooperative investigations of the Crops Research Division, ARS, U. S. Department of Agriculture, and the North Carolina Agricultural Experiment Station; published with the approval of the Director of Research as Paper No. 943 of the Journal Series.

[2] Pathologist, Crops Research Division, ARS, U. S. Department of Agriculture.

[3] Jenkins, M. T., and Alice L. Robert. 1952. Inheritance of resistance to the leaf blight of corn caused by Helminthosporium turcicum. Agron. J. 44: 136-140.

[4] Robert, Alice L. 1952. Cultural and pathogenic variability in single-conidial and hyphal-tip isolates of Helminthosporium turcicum Pass. U. S. Dept. Agr. Tech. Bull. 1058. 18 p.

[5] Luttrell, E. S. 1958. The perfect stage of Helminthosporium turcicum. Phytopathology 48: 281-287.

Results were best with small pieces of mycelium of compatible isolates on opposite sides of sterilized barley straw partially embedded in Sachs' agar in Petri dishes.

The present paper describes materials and techniques that have given abundant and consistent perithecial production, and presents evidence on the inheritance of compatibility factors. Perithecia were produced initially in matings of isolates 1170 and 1171, obtained from Luttrell, and were produced simultaneously in matings of 2 cultures of *H. turcicum* isolated from corn in western North Carolina. Also used were 1 culture from the Centraalbureau voor Schimmelcultures, Baarn, Holland, and 32 monoconidial isolates from naturally diseased corn leaves: 15 from North Carolina, 7 from Georgia, 2 from Virginia, and 1 each from South Carolina, Florida, Mississippi, Kentucky, Missouri, Minnesota, Iowa, and Wisconsin.

Perithecial production was abundant and consistent with the following technique: Barley grains, sterilized about 2 hours at 180°F dry heat in an oven, were partially embedded in Sachs' agar plus 20 g dextrose and 10 g cellulose gum per liter of agar. Isolates were mated by placing small agar blocks of mycelium on opposite sides of the barley grains. All matings were incubated continuously at 75°F. Immature perithecia appeared in 6–7 days, and mature ascospores were formed in about 20 days. Perithecia were usually abundant on the exposed portion of barley seed.

Results were also satisfactory with barley grains sterilized by autoclaving or with propylene oxide. Perithecia also developed on sections of sterile corn leaves on the medium, but they were frequently fewer than on barley grains, and seldom yielded as many ascospores.

The 33 monoconidial isolates, mated to tester lines, were found to belong to 1 of 2 compatibility groups, here designated *A* and *a*: 20 in group *A* and 13 in group *a*. Respective grouping was 8 and 7 for the North Carolina isolates and 4 and 3 for the Georgia isolates. Both mating types are present in a single area: both were obtained in corn fields less than ½ mile apart in western North Carolina.

The compatibility of monoascosporic isolates from conidial and ascosporic matings was studied. Of 218 isolates obtained from 5 different conidial matings, 112 belonged to *A* and 160 to *a*. The same ratio was found for 210 monoascosporic isolates obtained from 6 different ascosporic matings: 108 in *A* and 102 in *a*. Segregation of ascosporic isolates obtained from all conidial and ascosporic crosses totaled 220 *A* and 208 *a*. The number of ascospores isolated from any single cross ranged from 8 to 92. The segregation of isolates from any cross never deviated appreciably from the 1:1 ratio.

These results indicate that *T. turcica* comprises 2 compatibility groups and the compatibility reaction is controlled by a single major gene locus. Apparent differences in degree of compatibility between isolates have been observed, similar to those reported by Luttrell.[5] However, the differences were frequently minimized when suitable tested strains and the proper medium were used.

Certain reactions indicate that the sexual process is controlled by a number of gene loci. Six ascosporic isolates produced fertile crosses with tester strains of both compatibility groups, suggesting the presence of secondary genes or multiple alleles at the compatibility locus. Two isolates produced a few perithecia when selfed. Studies are in progress with these and other variants.—North Carolina State College, Raleigh.

A Leafspot of St. Augustine Grass Caused by Cercospora fusimaculans.[1] T. E. FREEMAN.[2] In the early spring of 1957 St. Augustine grass (*Stenotaphrum secundatum* (Walt.) Kuntze) was found affected by a leafspotting disease unlike diseases common on this grass in Florida. The disorder was initially found in the Gainesville area; however, continued observation throughout the summer revealed that it was widespread over the entire State. In addition, Luttrell[3] found apparently the same disease as far north as Griffin, Georgia. In many of the cases observed, damage caused by the leafspot was severe enough to concern those interested in propagating St. Augustine grass, and a study seemed desirable.

The symptoms consist of dark brown to purple oblong spots on the sheath and leaf blades. Young spots are uniformly colored, but tend to develop a tan center as the disease develops (Fig. 1). Lesions are usually distinct, but some coalescence may be noted in severe cases. Spots are 1–3×0.5–1 mm in diameter. Severely affected leaves become chlorotic and eventually die, leading to the gross symptom of an over-all thin turf. At certain stages these leafspots slightly resemble those of the commonly occurring gray leafspot disease of St. Augustine grass caused by *Piricularia grisea* (Cke.) Sacc. However, close examination shows that the two leafspots are dissimilar in shape, size, and color. Those caused by *P. grisea* are usually larger, more oval, and lighter brown than those of the disease reported herein. The casual observer has unknowingly confused the two entirely different diseases. In fact, packet F43660 in the Florida Agricultural Experiment Station Herbarium, labeled as containing St. Augustine grass affected by *P. grisea*, actually contained this grass affected by the disease reported herein. This mislabeled material was collected in Gainesville in November, 1952.

Microscopic examination of the leafspots revealed that a species of *Cercospora* was consistently associated with the disease. During moist weather, sporulation of this fungus was so abundant that the center of the spots appeared to have a whitish sheen. To deter-

[1] Florida Agricultural Experiment Station Journal Series, No. 766.

[2] Assistant Plant Pathologist, Department of Plant Pathology, Florida Agricultural Experiment Station, Gainesville.

[3] Personal correspondence dated August 13, 1957.

FIG. 1. Leafspot of St. Augustine grass caused by *Cercospora fusimaculans* Atk.

mine whether or not the *Cercospora* sp. was the causal organism of this disease, isolation and inoculation studies were carried out.

Numerous isolations from diseased material frequently yielded a *Cercospora* sp. The fungus grew slowly on Difco potato-dextrose agar fortified with 0.5% yeast extract; however, isolates sporulated frequently, though inconsistently. Spores produced by the isolates closely resembled those produced on the host. When St. Augustine grass was inoculated with conidial suspensions of the *Cercospora* sp., leafspots typical of those noted in the field developed within 10–14 days. Inoculations carried out with conidial suspensions both from sporulating pure cultures and from naturally diseased material resulted in leafspots; inoculations with suspensions containing only mycelial fragments did not. The *Cercospora* was readily isolated from leafspots produced by artificial inoculations. These isolation and inoculation data indicate that the *Cercospora* sp. is the causal agent of the disease studied.

A check of the literature revealed only one report of a *Cercospora* sp. on St. Augustine grass. Ogilvie,[4] working in Bermuda, briefly reported in 1923 that *S. secundatum* was attacked by a fungus of the genus *Cercospora*, which caused it to die in circular patches. He said the grass often was only slightly attacked. Only in shady situations did the fungus appear to do much harm. He also reported that the spores are produced on dark, linear areas of the leaves. In 1947 Waterston[5] included the *Cercospora* reported by Ogilvie in a check list of the fungi of Bermuda; however, neither Waterston nor Ogilvie gave a specific name. Chupp[6] did not mention *Cercospora* on *S. secundatum*. Since Ogilvie's report was only a brief note, with no description of the causal fungus and only a superficial description of the symptoms of the disease, it is impossible to say whether or not it is the same as the disease reported in this article.

The *Cercospora* sp. causing this leafspot of *S. secundatum* can be described as follows: Sporulation amphigenous, but more profuse on the upper surface; stroma small, usually filling the stomatal opening; fascicles rarely dense; conidiophores brown, slightly clavate, irregular near the conic tip, spore scar distinct, 0–2 septate, 20–43×3–4 μ; conidia hyaline to subhyaline, cylindric to slightly obclavate, straight to variously curved, sometimes catenulate, indistinctly 2–7 septate, base obconically truncate, tip usually blunt, 33–60×1.8–3 μ. With only minor exceptions, the characteristics of this *Cercospora* fall within those given by Chupp[6] for *Cercospora fusimaculans* Atk. Material collected in the Gainesville area was submitted to Chupp,[7] who agreed that this fungus fits the description of *C. fusimaculans*. Therefore, *C. fusimaculans* is considered to be the causal organism of this leafspot disease of St. Augustine grass. As far as the author can determine, this constitutes the first definite report of this species of *Cercospora* on a member of the genus *Stenotaphrum*.

A specimen has been deposited in the Florida Agricultural Experiment Station Herbarium (F46494).—Department of Plant Pathology, Florida Agricultural Experiment Station, Gainesville.

[4] Ogilvie, L. 1923. Preliminary report of the plant pathologist for the period September 27th to December 31, 1923. Repts. Board and Dept. of Agr. Bermuda 1923. p. 24-34.

[5] Waterston, J. M. 1947. The fungi of Bermuda. Dept. Agr. Bermuda Bull. No. 23. p. 238.

[6] Chupp, C. 1953. A monograph of the fungus genus Cercospora. Ithaca, New York. 667 p.

[7] Thanks are extended to Dr. Charles Chupp for verifying the identity of the fungus.

REPORT OF THE 50TH MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The American Phytopathological Society held its Golden Anniversary meeting in conjunction with the meeting of the American Institute of Biological Sciences, on the campus of the University of Indiana, at Bloomington, on August 25 through 28, 1958. Attendance of Society members, estimated at about 1200, was the largest ever gathered at an annual meeting. The program consisted of 9 symposia on viruses, fungicides, pathogenicity of fungi, parasitism, soil microbiology, and epidemiology, including speakers from various countries of the world; 6 major addresses (including the keynote address for the A.I.B.S. meetings) dealing with historical aspects of plant pathology, and 60 papers on original research.

Many elaborate and instructive exhibits were set up by the Society, individual institutions, and research organizations to celebrate the 50th birthday of the Society and 50 years' development of plant pathology.

The Council met on August 24 and 28, and, for this year only, a single general business meeting of the Society was held, on August 28, to conduct necessary business.

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REPORTS OF OFFICERS, COMMITTEES, AND REPRESENTATIVES

Report of the Secretary. The Council has adopted the practice of reporting the membership as of the last day of the year preceding the annual meeting. This will give a full account of membership and allow for annual comparisons on a definite date.

As of December 31, 1957, the American Phytopathological Society had 1901 members. New member applications, 166; regular, 52; student, 114; life members, 100. Since our preceding meeting the following members have died: S. L. Hopperstead, Jacob Peleg, Raymond F. Poole, John W. Roberts, A. B. Stout, Sam F. Trelease, Mary G. Van Meter.

WM. B. HEWITT

Report of the Treasurer and of the Business Manager of Phytopathology. The following report of Seward and Monde, Certified Public Accountants, New Haven, Conn., is given in lieu of the report of the retiring Treasurer and Business Manager:

Dr. Paul R. Miller, President
The American Phytopathological Society
Beltsville, Maryland

We have examined the Statement of Cash Receipts and Disbursements (Treasurer's Account) of The American Phytopathological Society for the fiscal year ended June 30, 1958. Our examination was made in accordance with generally accepted auditing standards and accordingly included such tests of the accounting records and such other auditing procedures as we considered necessary in the circumstances.

A schedule of the invested funds of the society is furnished as part of this report.

In our opinion, the accompanying Statement of Cash Receipts and Disbursements (Treasurer's Account) presents fairly the results of operations on a cash basis of The American Phytopathological Society for the fiscal year ended June 30, 1958.

/s/ Seward and Monde

Certified Public Accountants

New Haven, Connecticut
August 6, 1958

THE AMERICAN PHYTOPATHOLOGICAL SOCIETY
STATEMENT OF CASH RECEIPTS AND DISBURSEMENTS
(Treasurer's Account)
Fiscal year ended June 30, 1958

Receipts:		
Membership dues		$ 7,175.00
Sale of Fungicide Data Summaries		119.00
Contributions from industry supporting 50th Anniversary		8,525.00
National Science Foundation Grant G-4605, supporting 50th-Anniversary volume		13,000.00
Advance sales of 50th-Anniversary volume		5,057.10
Total		$33,876.10
Disbursements:		
Office of President	$ 98.60	
Office of Secretary	1,253.94	
Office of Treasurer	158.74	
Annual meeting expenses	1,111.29	
Fungicide data summaries	441.71	
Transfer of balance of fungicide data summary account to Dr. A. B. Groves	960.89	
Public Relations Committee expense	240.00	
Fiftieth-Anniversary Committee expense	25.76	
Representative to Iowa State College Centennial	39.20	
Fiftieth-Anniversary volume expense (applicable against NSF Grant G-4605)	562.21	

Fiftieth-Anniversary symposium expense (applicable against Rockefeller Foundation Grant)	118.33	
Production of Fiftieth-Anniversary motion picture	3,683.11	
Brochure and monograph expense	2,241.24	
Dues—American Institute of Biological Sciences—two years	3,710.00	
Contribution—Joint Committee on Grasslands Farming	10.00	
Refunds	25.50	$14,680.52
Excess of receipts over disbursements		19,195.58
Cash balance, July 1, 1957		11,277.77
Cash balance, June 30, 1958—forwarded		$30,473.35

PUBLICATION OF JOURNAL—PHYTOPATHOLOGY

Receipts:		
Subscriptions		$23,123.96
Advertisements		2,716.64
Sales of back issues		1,142.28
Reprints and excess illustrations		7,276.45
Sustaining associate memberships		1,300.00
Income tax refund		1,247.47
Interest and dividends on investments		1,121.53
Sale of American Telephone and Telegraph Co. rights		5.80
Total		$37,934.13
Disbursements:		
Printing and distribution of PHYTOPATHOLOGY	$24,617.59	
Cost of reprints	2,040.22	
Office of business manager	1,587.81	
Office of editor-in-chief	7,056.13	
Office of advertising manager	279.08	
Cost of new investments	284.68	
Collector of Internal Revenue	797.60	
Refunds	133.30	$36,796.41
Excess of receipts over disbursements		1,137.72
Cash balance, July 1, 1957		$26,518.55
Cash balance, June 30, 1958		$27,656.27
Total cash balance, June 30, 1958		$58,129.62

SINKING FUND

		Book Value
Bonds		
$2,500	United States Savings Bonds, Series "J"	$ 1,800.00
425	United States Savings Bonds, Series "F"[a]	313.50
Stock		
80 shs.	American Cyanamid Co.	2,160.47
7 shs.	Family Finance Corp. (acquired by gift; nominal value)	7.00
57 shs.	Johns-Manville Corp.	1,946.90[a]
60 shs.	Standard Oil Co. of Ohio	2,018.65
22 shs.	Westinghouse Electric Co.	849.08
Savings Accounts		
	Northwestern Federal Savings and Loan Association	2,000.00
	New Haven Savings Bank	216.55
	Total	$11,312.15

[a] $400 United States Savings Bonds Series "F" matured in 1956 and have not been redeemed.

LYMAN MEMORIAL FUND

Stock		
35 shs.	Westinghouse Electric Co.	$ 1,350.81
22 shs.	American Telephone and Telegraph Co.	3,282.15
	Total	$ 4,632.96

PHYTOPATHOLOGY CONTINGENCY FUND

Stock		
14 shs.	Johns-Manville Corp.	$ 466.00
Savings Accounts:		
	Arlington and Fairfax Savings and Loan Association	7,872.95
	Prudential Building Association	6,819.55
	Arundel Federal Savings and Loan Association	9,077.81
	Southern Federal Savings and Loan Association	9,695.43
	Decatur Federal Savings and Loan Association	8,561.85
	Fidelity Federal Savings and Loan Association	7,997.43
	Southwest Savings and Loan Association	7,983.32
	Lawn Savings and Loan Association	8,000.00
	Total	$66,474.34

FIFTIETH ANNIVERSARY FUND

Savings Account:	
Standard Federal Savings and Loan Association	$ 9,637.64
Total of all invested funds	$92,057.09

Report of the Auditing Committee. The Auditing Committee accepts the audit report of the accounts of the American Phytopathological Society and of Phytopathology for the fiscal year 1957–58 as submitted by Seward and Monde, Certified Public Accountants, New Haven, Conn., on August 6, 1958.

PAUL E. WAGGONER
P. M. MILLER
A. E. DIMOND

Report of the Editor-in-Chief. On December 31, 1957, after 3 years of distinguished service to the Society, Dr. A. Frank Ross retired as Editor-in-Chief. With his help and guidance the new Editor-in-Chief was initiated into the job, and the files were transferred from New York to California without a hitch. In a departure from past practice, a professional editor was hired on a part-time basis. We were fortunate in obtaining Mr. Kelvin Deming, University of California editor stationed at Davis. His outstanding abilities and the help of an efficient secretary have lightened the task of the Editor-in-Chief.

In his 1957 report, Dr. Ross could not include material published in the November and December issues of Volume 47. These issues contained 25 technical articles, 2 obituaries, 4 Phytopathological Notes, 4 book listings, 1½ pages of news, 8½ pages of advertising, and 22 pages of index (including a brief list of errata). During the last 5 months of 1957, 4 articles and 2 Phytopathological Notes were rejected or withdrawn.

It is estimated that Volume 48 will contain 710 pages. At the time this report is being written (July 1–10), 6 issues have been published, the July issue is in the page-proof stage, and the dummy of the August issue has been sent to the printer. The first six issues contain 67 technical articles (312 pages), 9 Phytopathological Notes (10 pages),

60 abstracts of Divisional meetings (11 pages), the report of the 49th annual meeting (11 pages), 1 obituary (1 page), and 8 book listings (1½ pages). During the first six months of 1958, 108 manuscripts (1457 typewritten pages) were received. During this time, 4 technical articles, and 4 Phytopathological Notes were rejected or withdrawn.

A 72-page Directory, distributed with the May issue of Phytopathology, lists the names, addresses, and fields of interest of the 1902 members of the Society. Dr. S. E. A. McCallan did a splendid job of assembling the material, compiling the information for the tables and geographical index, and writing the introduction. Only the editing and preparation of the manuscript for the printer were done in this office.

Processing an article in Phytopathology can be divided into two phases: 1) preparation for printing, and 2) printing. When a manuscript is received it is recorded and sent to a reviewer who is familiar with the subject matter. The reviewer reads the manuscript, paying particular attention to the experimental methods and results, and to the manner in which the author has presented the data and arrived at the conclusions. He verifies the literature citations. He prepares a report recommending acceptance or rejection. If the article is acceptable, he suggests any changes he considers necessary. The manuscript is sent back to the editorial office, where it is examined for clarity, sentence structure, grammar, quality of illustrations, and arrangement of tabular material. The manuscript, together with the report of the reviewer and the report of the Editor-in-Chief, is then returned to the author. When the author has made the necessary changes and returned the manuscript, it is accepted for publication. This is the end of the first phase. The second phase begins when the manuscript is marked for the typesetter and sent to the printer. The printer returns galley proof, which is sent to the author for proofing. The next step is the preparation of a dummy from the galley proof. This dummy guides the printer in making up the issue. Before final printing, however, page-proof of the issue is furnished the editorial office for final proofing.

During the first four months of 1958, the average time required for the first phase of handling manuscripts (receipt to acceptance) was 58 days: 23 days for reviewing, 6 days for editing, and 29 days for revising. The time required for the second phase (acceptance to publication) averaged 160 days: 50 days for typesetting, 12 days for authors to proof galleys, 29 days for make-up, 29 days for printing page-proof, and 36 days for final printing. Spot checks of the time between acceptance and publication in other years gives an average of 151 days. The 9-day difference reflects a greater distance than in former years between the editorial office and the printer and most sources of manuscripts.

In conclusion, I wish to express appreciation to the Editors for critical and constructive evaluation of manuscripts; to Mr. John Ferguson, III, and his staff at Monumental Printing Company for cooperation and skill in meeting the many problems of publication; to Dr. Saul Rich for efficient handling of business matters; and to officers and members of the Council for their interest and support.

E. E. WILSON

Report of the Advertising Manager. There were 57.5 pages of paid ads, 8.5 pages of News, 2 pages of Phytopathological Classics ads, and 6 pages of free ads that appeared in the 12 numbers of Phytopathology issues between July 1, 1957 and June 30, 1958. Financial accounting of the Advertising Office is in the report of the Business Manager.

Grateful acknowledgment is made to A. F. Ross, former Editor-in-Chief, to E. E. Wilson, Editor-in-Chief, and to Saul Rich, Business Manager, for their assistance in matters relating to advertising in Phytopathology. The cooperation of F. L. Brown, Monumental Printing Company, is sincerely appreciated.

Special thanks are due to C. H. Ward, who is ably managing the office during the leave of absence of the Advertising Manager.

D. A. ROBERTS

Report of the A.P.S. Representative, Board of Editors, American Journal of Botany. The representative on the Board of Editors has received no communications from the editorial staff of the American Journal of Botany.

A. F. Ross

Report of the A.P.S. Representative, A.I.B.S. Board of Directors. This is the final report of the retiring representative after four years of participation in the reorganization and rapid growth of the Institute. The membership, the Council, and the Officers of the American Phytopathological Society have shown outstanding comprehension of the problems involved, and commendable leadership in the support of corporate action of the biological sciences through the Institute. Had all other biological societies shown similar insight and rendered similar support, the biological societies combined might have played a more important role in the present initial stages of reorganization of science in government, and they might have had more biological representation on national advisory committees. That, perhaps, is idle wishing, for the concept of strength through corporate action is still too immature in the minds of biologists in general for effective effort in the present reorganization of science and education on a national scale.

Other branches of science with years of experience in coordination of thought and effort into corporate action have demonstrated its strength and effectiveness. The biologists in general must study these examples and appreciate the significance of strength through union before biology and biologists are prepared to render full service in the national development of research and education. The following statement represents my attempt to formulate the problem and define the value of corporate action in biology.

Strength under the democratic system rests in the intelligent embodiment of individual thought and effort into corporate action in specific fields of endeavor. The democratic process of resolving individual ideas into a wise plan of action commonly acceptable results in sound judgment effectively presented. The full realization of the importance of this united action on the part of the biologist is essential to the development of biology and to national welfare in this period of educational and scientific reorganization on a national scale.

The segregation of biologists under diverse disciplines and interests, as expressed through the numerous scientific societies, has dissipated corporate strength in a period when coordinated thought and action are essential to national progress. The scientific society with membership of a few thousand is no longer effective alone in influencing and directing representative progress. The combined coordinated effort of some sixty thousand biologists can be effective, not only in national guidance of biological science and education but also in the constructive expression of individual thought and the development and use of personal potential.

The significance of the scientific disciplines to education and national development and national welfare is going through a period of self-analysis and national evaluation. The great danger threatening future progress is that national legislation is prematurely enacting science reorganization and financial support, without full representation of science and scientific thought. Progress in wisely and constructively projecting science, scientific attainment, and technological development, as well as education, involves study and thought contributed by all scientists. Effective and efficient use of the large sums appropriated for the

support of education and research, and wise use of grants for the support of individual research, travel, and education, all rest on coordinated study and evaluation of over-all national needs and the wise support of individual research and development in all fields of science.

The strength expressed through corporate action in some fields of science is illustrated by the phases of scientific endeavor presently emphasized in the national program and in the scientific personnel appointed to the national planning boards and advisory committees. Biology, the science dealing with the development, nutrition, and health of plants and animals; biology, the basic science determining human behavior, is largely omitted from the national program, represented only by a small minority of biologists on the advisory boards. The biologists themselves are principally responsible for this minority representation in planning for the future.

The biologists are the last large group of scientists to recognize the importance of, and the need for, coordinated thought and action in this period of rapid scientific development. United action is essential for adequate biological representation on national science advisory committees. Coordination of policy is necessary in supplying information to legislative committees for use in wise enactment of national legislation dealing with biology. Now is the time for each biologist to come to the aid of his country through personal support of society membership in the biological institute.

The full realization of corporate strength and democratic representation of biologists rests upon all biological societies becoming members with voting power on the governing board of the American Institute of Biological Sciences. Adequate financial support from all biologists, contributed through membership dues, is a second essential. For the effectiveness of corporate action, the expansion of activities and the services rendered the individual are determined by the support contributed by the individual biologist. The rightful place of biology in the national development of science and education is contingent on this joint support. Progress in protecting the rights, interest, participation, and development of the individual, our greatest asset, rests on full support of wisely coordinated action through institute expression.

The institute has grown in stature and in the direction of biological thought on a national scale in spite of the handicaps enumerated. These advances have occurred largely through the active support of the governing board and the able, intelligent, and dynamic efforts of the Executive Director, Dr. Cox, and his small staff. Unfortunately, fewer than half of the biologists of the nation have representation on the governing board. Additional large-membership societies have increased their dues voluntarily to include the full membership, i.e., they have waived the one-thousand-dollar membership limit. Several new societies, including a few with large numbers, have voted to become members with the payment of full membership dues. Notable among these is our sister society, The American Society of Entomologists. A number of additional societies representing important sections of biology have voted to become affiliates. The growth and support have been encouraging, although much too slow to keep pace with the significant requests presented to the Institute by government agencies and the general recognition of the Institute as a clearing house for biological information. Some of the major accomplishments are summarized:

1. The services rendered and the assistance given by A.I.B.S. in securing financial support for the Semicentennial Meeting of the American Phytopathological Society are known to the Council and Officers. The members should be advised of the assistance given in securing travel grants, funds for publication of symposia, and other support in connection with preparation for the annual meeting in 1958.

2. A.I.B.S. supervises, under contract, the operation of the National Register of Scientific and Technical Personnel. With the approval of the contracting agency, the data contained in these records were analyzed, and the information on the national status of biologists as compared to other scientists was presented to congressional committees, government agencies, and the public. All biologists have felt the impact of this information, but especially those under Federal Civil Service.

3. The requests from legislative committees for information on the role of the biological sciences in national planning and development are swamping the small staff operating on the meager budget supplied by the biologists. A.I.B.S. is recognized and accepted as the source of information in biology.

4. Government agencies distributing funds for research, education, and travel are asking A.I.B.S. to arrange for study committees in the field of biology as well as to contract to perform specific studies and other projects. Some ten contracts of this nature are in operation this year, including added survey and translation of publications in foreign languages. These contracts and the amounts involved are shown in the budget summary.

The brief summary of the budget illustrates the very meager financial support from the biological societies in contrast to the services rendered the societies and biology. As stated earlier, the adequate financial support of all biologists contributed through membership dues is necessary for the healthy growth and corporate expression of biology. Moving in this direction, the governing board voted 1) to rescind the one-thousand limit on membership dues, and 2) to place affiliate dues on a membership basis of determination, i.e., increasing the amount paid on a membership ratio. These changes were approved in the belief that the services to biologists cannot be soundly financed from operating costs assessed to contracts made for rendering specific services.

A brief analysis of the operating budget of A.I.B.S. supports the introductory statement above and the general theme of this report to the Society. During the calendar year 1957 the A.I.B.S. operated as a business with a gross of about $400,000. In 1957 the total income from dues—membership, affiliate, and associate—was less than $22,000. By contrast, about $33,000 was collected in overhead from contracts and grants. These overhead funds were applied to the general operation of the institute. Stated on a ratio basis, exclusive of contract-committed money for specific purposes, the Institute collects for its own uses $1.50 for every $1.00 in dues from members. The Institute, from its initiation, has never maintained itself from dues of societies. This ratio of operational dollars amply illustrates the inadequate support from the individual biologist. In contrast, if each member of each biological society were paying his way ($1.00), this income from dues would be well over $50,000. Is there a better answer than this to the question of the reason for the minority representation of biology in the national program?

JAMES G. DICKSON

Report of the A.P.S. Representative, A.A.A.S. Council. As representative of the Society on the Council of the A.A.A.S., I voted for the election of Paul E. Klopsteg as President-elect, and for William W. Ruby and Thomas Park for four-year terms on the Board of Directors. Unfortunately, I was not able to attend the annual meeting of the Association this year.

Probably the most significant project of the A.A.A.S. for the year was the "Parliament of Science," held in Washington on March 15, 16, and 17. As a member of the Council, I was invited to nominate people to attend the meeting. I nominated 26 people in the field of Botany. This included the following plant pathologists: A. E. Dimond, James H. Jensen, Arthur Kelman, G. L. McNew, Glenn S. Pound, E. C. Stakman, Russell B. Stevens.

I was unable to go, because of a conflict with a meeting of the Advisory Committee on Biology and Medicine to the Atomic Energy Commission. The proceedings of the Parliament of Science were published in SCIENCE in the spring.

JAMES G. HORSFALL

Report of the A.P.S. Representative, Division of Biology and Agriculture, National Research Council. The Division of Biology and Agriculture met with the National Research Council, and the general program was discussed except for one meeting. A seminar on teachers in biology brought out that higher salaries would attract better people. Considerable indecision was expressed as to the usefulness of revising the "Handbook of Biological Data."

S. J. P. CHILTON

Report of the A.P.S. Representative, Agricultural Research Institute. This report covers events that transpired at the sixth annual meeting of the American Research Institute, held in Washington, D. C., October 14 and 15, 1957, and of special committee meetings since that time. An item of interest to plant pathologists was the adoption of a recommendation whereby the President of ARI would appoint an *ad hoc* committee to investigate ways and means of initiating a public relations program for agriculture and agricultural research. This committee met on January 23, 1958, and will report on plans to the full membership of ARI.

As representatives of the American Phytopathological Society, we wrote a letter to the President on May 20, 1958, saying that our society favored the implementation of an agricultural public relations program and suggested that this committee contact the publicity chairman of our Society.

At the Agricultural Board meeting held April 25 and 26, 1958, a letter was read from the Rockefeller Foundation making available $9500 to the National Research Council in support of a study on agricultural losses caused by plant pests. This work will be undertaken by the Council's Committee on Agricultural Pests. The committee met at Beltsville, Maryland, July 14 and 15, and formulated methods and plans for obtaining and analyzing information on agricultural losses. The Agricultural Pest Committee is also soliciting suggestions from the subcommittees on related problems needing research. Reports from these groups should be forthcoming during the year.

The Seventh Annual Meeting of ARI is scheduled for October 12, 13, and 14, 1958, in Washington, D. C.

ERWIN L. LECLERG
C. L. LEFEBVRE

Report of the A.P.S. Representative, Food Protection Committee, Food and Nutrition Board, N.A.S.–N.R.C. The Seventh Annual Meeting of the Liaison Panel of the Food Protection Committee, held in Washington, D. C., December 12–13, 1957, was devoted to an orientation conference on carcinogenesis. This consisted of a general discussion of definitions and methods. None of the materials mentioned were directly related to plant pathology.

Other activities and publications of the Committee during 1957 were primarily concerned with chemical additives in food processing and food packaging materials, also matters of no direct interest to plant pathologists.

J. R. MCGREW

Report of the A.P.S. Representative, American Type Cultural Collection. A certified audit of the Collection's finances, December 31, 1957, showed total assets of $110,255.21. After deduction of the amounts obtained from grants for research, the assets of the Collection amount to $96,995.21. The income from the sale of cultures in 1957 was $53,183.25. This is an increase of nearly 13% over sales in 1956. Culture sales during the first six months of 1958 amounted to $26,500, which is slightly more than a 10% increase over the same period in 1957. The reserve fund obtained through the National Research Council has increased from $7,848.72 to $8,728.26. Our equity in the building we occupy amounts to $21,889.30, as of December 31, 1957. This represents slightly more than 56% of the total valuation of the property.

The Naval Research grant terminates in August 1958. This grant made it possible to lyophilize over 1000 bacterial strains that are of special importance. Two cultures of each strain are stored under refrigeration at the Armed Forces Institute, and three cultures of each are stored at room temperature at the ATCC. As soon as facilities are available, these cultures will be stored under refrigeration in our building. These are all reserve cultures, independent of the regular stock cultures from which orders are filled. The Collection will continue this program with important strains of the molds and yeasts.

Applications have been submitted to the National Institutes of Health for grants during 1959 and 1960 for the continuation of Dr. Clark's (Assistant Curator) researches on the classification of bacterial viruses. His research in this field has been supported by the NIH for two years. A temporary subsidy of $1000 was obtained from the Rockefeller Foundation for the program on animal and human viruses, pending the receipt of support for the extension of this program for which application has been made to the National Institutes of Health. This application is for $5000 for the first year, with authority to extend support for five years.

The general catalogue of cultures is in the hands of the printer. Publication will be in August or September. This catalogue will be sold for $1.00. There have been unavoidable delays in revision of the two virus catalogues. It is expected that the cost of the new animal virus catalogue will be covered by the NIH grant, and the catalogue will be distributed free of charge. The revision of the plant virus catalogue will consist of supplementary pages. These will be distributed free of charge. Since the income from plant virus cultures is small ($34.11 in 1957) it is hoped that the Society will see fit to contribute $200 toward defraying the expense of this revision.

The salary scale for staff members of the Collection is comparatively low, about 12½% under the new Government salary scale. This constitutes a problem in Washington. We have a group health plan in operation, but as yet we do not have a retirement plan for the entire staff.

H. H. MCKINNEY

Report of the A.P.S. Representative, Research and Development Associates, Food Container Institute. Your representative has kept in touch with the Research and Development Associates, Food and Container Institute for the Armed Forces, throughout the past year. The Activities Reports were received and studied. The annual meeting was held in Chicago, April 22 to 24. Among the most important food and packaging projects for the Armed Forces were those on radiation preservation of food and freeze dehydration. A typical freeze-dehydrated army meal was prepared and served within a few minutes at one meeting.

Dr. F. P. Mehrlich was appointed Scientific Director of the Quartermaster Food and Container Institute. He succeeds Donald K. Tressler, who resigned in February.

Working through a contract with the Quartermaster Food Preservation Project, your representative and his associates have continued studies on control of postharvest diseases of fresh fruits and vegetables by means of gamma radiation. The Quartermasters are particularly interested in the possibility of irradiating citrus fruits at dosages sufficient to control or retard decay under the conditions of oversea shipping at their disposal. Up to the present time, significant control of the following diseases has been obtained by proper dosages of gamma radiation without injury to the commodity: peaches, brown rot and Rhizopus rot; oranges, Diplodia and Phomopsis rot; lemons, blue and green mold rot; strawberries, Rhizopus and Botrytis rot; grapes, Botrytis rot.

It is recommended that a representative be appointed for 1959.

G. B. RAMSEY

Report of the A.P.S. Representative, Biological Stain Commission, Board of Trustees. My insistence of exact identification of stains and unusual chemicals, as

proposed last year, was truly justified and graciously accepted by several authors in Phytopathology. This year I observe nothing new for the subject except the insistence of our Commission that a truer Color Index Value or Number be arrived at and agreed upon for standard stains. This insistence does not appeal to me, since indexes will vary with the manner in which the dyes are used. Therefore I have no new data to propose.

FRANK P. MCWHORTER

Report of the A.P.S. Representative, American Standards Association Sectional Committee on Common Names for Pest Control Chemica's, K62. During the past 12 months, 9 common names have been officially accepted: the herbicides monuron, diuron, erbon, neburon, dalapon, silvex, fenuron; the miticide ovex; and the acaracide and insecticide ethion. Proposed common names for two systemic insecticides and a rodenticide are being studied. No fungicide names are now under consideration, because none have been proposed by the manufacturers or others interested.

It should be emphasized that it is not simple to select a common name. The great majority of coined names that at first were thought suitable are either already used for another product or are too similar to the name of another product to be acceptable. A meeting was held at the A.S.A. headquarters in New York City on November 7, 1957, to clarify the rules regarding the degree of similarity that could be tolerated in proposed common names. The rules were liberalized, yet the similarity must not cause confusion primarily between agricultural chemicals, pharmaceuticals, and foods.

The International Organization for Standardization met in London, September 18–20, 1957. Delegates attended from 7 countries, and observers were present from 3 countries, from the International Bureau for the Protection of Industrial Property, and from the World Health Organization.

Committee K62 was represented by the Chairman, Dr. H. L. Haller. The name of the international committee, ISO/TC81, has been changed to "Common Names for Pesticides." Efforts are continuing toward international agreement on common names and on rules pertaining to their use.

Continued representation by The American Phytopathological Society on A.S.A. Sectional Committee K62 is recommended.

S. E. A. MCCALLAN
W. D. MCCLELLAN

Report of the A.P.S. Representative, American Grassland Council (formerly Joint Committee on Grassland Farming). Under the new name this organization feels it can wield more influence in getting support for grassland research. Four classifications of membership (and dues) have been established: Individual, $5.00; Professional or Scientific Society, $10.00; Trade and Business Association, $25.00; Company or Corporation, $100.00.

In keeping with our past relationship with this organization, I feel that the American Phytopathological Society should perhaps continue representation in it, and suggest that the Society join the American Grassland Council and designate someone to represent our Society in the organization.

K. W. KREITLOW

Report of the A.P.S. Committee on Extension. The entire committee has not met formally this year; however, several members have met informally, and correspondence contacts have been maintained. The committee sponsored two meetings for extension workers during the 1957 A.P.S. meetings at Palo Alto. At one session, extension problems of mutual interest were informally discussed. The evening session, attended by 58 persons, included a panel discussion on methods of improving relations with and between county and state extension personnel, processors, farmers, and chemical fieldmen. Various aspects of establishing and maintaining plant disease clinics were discussed during the latter portion of the program.

The major effort of the committee in 1958 has been the compilation and selection of outstanding 2×2 color transparencies of plant diseases for duplication and sale to agricultural workers. Slide sets for 7 crop categories, each consisting of 24–54 slides, have been prepared, complete with syllabi, and will be offered for general sale at a nominal price this fall. These should be valuable aids for vocational agricultural teachers, 4-H workers, commercial agricultural fieldmen, and county agents. Another continuing project has been the investigation of new, lower-cost color printing processes for bulletins. The committee prepared a large exhibit, entitled "Extension—A Vital Facet of Plant Pathology," as a part of the 50th Anniversary Exhibition, at Bloomington.

The committee sponsored an informal breakfast for all extension pathologists at the Bloomington meeting.

ARDEN F. SHERF, *Chairman*

Report of the Committee on Finances and Investments. Total invested funds, $93,264.50, earned $3,803.47 in the year ending June 30, 1958, a yield of 4.19%. The yield in the preceding year was 3.56%.

The following table summarizes our investments and earnings for the past fiscal year:

Investment	*Total*	*Earnings*	*Yield–%*
Insured Savings and Loan Accounts	$78,041.89[a]	$2,980.18	3.95
Common stock (original cost)	12,081.06[b]	729.20	6.04
U. S. Savings Bonds (at maturity)	2,925.00	87.75 (est.)	3.00
New Haven Savings Bank Account	216.55[a]	6.34	3.01
Totals and average yield	$93,264.50	$3,803.47	4.19

[a] Includes accrued interest and dividends.

[b] At close of market July 9, 1958, our common stock was worth $16,697.50.

Transactions for the past fiscal year:

1) American Cyanamid common stock split 1 for 1, raising our holdings from 40 to 80 sh.
2) Bought 2 sh of Amer Tel & Tel for $284.68, plus rights.
3) Interest and dividends allowed to accrue in pass-book accounts.

During the year, the Internal Revenue Service ruled that we are not required to pay any income tax on dividends and interest. They refunded to us the $1,128.77 we had paid, plus interest of $118.70, for a total of $1,247.47.

A. E. DIMOND, *Chairman*

Report of the Standing Committee on History of Plant Pathology. The historical records of the Society maintained by the Committee on History of Plant Pathology have been in active service during the year under review. They were drawn on freely in the preparation of a paper by the Chairman on the early history of phytopathology in America. A limited number of items have been added to the collection during the year.

JOHN A. STEVENSON, *Chairman*

Report of the Committee on International Cooperation. Some of the members of the Committee have been active in helping with the program of the IV Reunion Latinoamericana de Fitotécnia, to be held in Santiago from November 24 to December 6, 1958, at which there will be a good phytopathological program, particularly emphasizing the need of cooperation in Latin America and, to a certain extent, the hemisphere. It is hoped that a closer organization of pathological activities on the part of this organiza-

tion may facilitate necessary cooperation with U. S. pathologists.

The Chairman of the Committee attended the IV International Congress of Crop Protection, in Hamburg, Germany, in September 1957, as did a number of other North American phytopathologists. Arrangements were made for regional international cooperation in certain specific problems. The most important question raised at the Congress, however, was the possibility that the V Congress, scheduled for 1961, might be held in the United States. It would seem very desirable that the Congress should be held here, but preliminary inquiries have not been very encouraging. If the A.P.S. wants to take its international program seriously, it is suggested that they might well take the leadership in at least exploring all possibilities of holding this Congress in the United States in 1961.

It is hoped that there will be opportunity for a meeting of at least a quorum of the Committee at the Bloomington meetings. At this time policies should be discussed and a definite program of activities should be undertaken. It is suggested, therefore, that consideration then be given primarily to two things: 1) the possibility of holding the V International Congress of Crop Protection in the United States in 1961; 2) the planning of a long-time program with a view to its adoption at the annual meeting of the Society in 1959. The Committee will assume as its responsibility the formulation of a suggested program. In the meantime, a number of members of the Committee have been doing what they can to promote international cooperation.

Unfortunately, as is often true, this report has not yet been submitted to the full membership of the Committee; but attempts will be made to hold a meeting early during the sessions at Bloomington.

E. C. STAKMAN, *Chairman*

Report of the Committee on Phytopathological Classics. On the occasion of the Society's Golden Anniversary Celebration, the Committee on Phytopathological Classics presents its tenth Classic. PC No. 10 features two epoch-making papers in the history of pathogenesis by a pioneer pathologist who helped overthrow the theory of spontaneous generation and establish the germ theory of disease—Agostini Bassi (1773-1856). As pointed out by Steinhaus: "The importance of Bassi's work has to a great extent been overshadowed by that of Pasteur's a few years later, but his genius deserves greater recognition than it has received. It was Bassi whose discoveries ushered in the dawn of the science of infectious disease. Certainly he stands as a giant among those early workers who may be said to have laid the first foundations for insect pathology."

The translation, based on the 1835 edition of Bassi's "Del mal del Segno," has been prepared by P. J. Yarrow, of the University of Exeter. An appreciation of Bassi de Lodi is contributed by G. C. Ainsworth and the translator.

The report of the Treasurer of Phytopathological Classics, Dr. Paul H. Wooley, for the fiscal year July 1, 1957, through June 30, 1958, is as follows:

Receipts:				
Cash balance on July 1, 1957			$1,728.23	
Received during fiscal year 1957-58:				
From sale of Classics		$ 91.50		
Originals	$76.50			
Reprints	15.00			
Accounts Receivable 1957-58		10.25		
Originals	8.75			
Reprints	1.50		101.75	$1,829.98
Disbursements:				
Refunds on orders for PC #3		2.00		
Cash balance on June 30, 1958		$1,827.98		$1,829.98

The number of copies of Phytopathological Classics sold during the fiscal year was 92, having a total value of $100.00. Numbers of CLASSICS on hand at Ithaca, New York, and at Lake Alfred, Florida, on June 30, 1958, were: No. 1, 468; No. 2, 374; No. 3, 0; No. 4, 79; No. 5, 302; No. 6, 349; No. 7, 281; No. 8, 463; and No. 9, 677.

L. C. KNORR, *Chairman*

Report of the Committee on Phytopathological Monographs. The committee has continued its efforts to obtain manuscripts on diseases suitable for monographic treatment. Promises of manuscripts for 13 diseases have been obtained. No manuscripts have yet been forwarded to the committee. Timing on at least four manuscripts would indicate possible publication in 1959.

Only one author has requested financial assistance on a monograph. The assistance requested was provided by the council which asked that in the future each author make a full statement of needs for completion of the manuscript at the time of the initial request for assistance.

G. C. KENT, *Chairman*

Report of the Editor of News. With the June issue of PHYTOPATHOLOGY we celebrated our 10th anniversary of publishing a News Page. After numerous misgivings and much advance preparation, the first regular page of news items was assembled, printed, and mailed separately to all members, in June, 1948. Thereafter, until January, 1951, the News Page was prepared bimonthly and mailed to members. Since January, 1951, the News Page has become a regular part of each number of PHYTOPATHOLOGY, usually appearing on the last page. Since early 1956, alternate half and full pages of news items have appeared in each number of the Journal.

The editor wishes to acknowledge his appreciation to members of the Standing Committee for Preparation of the News Page for their able assistance in obtaining and forwarding news items. During the past year the Committee consisted of the following: L. O. Weaver, Shirl O. Graham, D. E. Ellis, S. H. Davis, Roy D. Wilcoxson, and E. J. Hawn.

K. W. KREITLOW

Report of the Committee on Phytopathological Reviews. The Committee on Phytopathological Reviews has endeavored this year to make a positive beginning toward establishing the program of review articles that was authorized some time ago. Initially, we compiled as comprehensive a list as we conveniently could of those articles which it appeared were already in some stage of preparation for outlets other than Phytopathology. This list totaled well over 100 and included material being readied for the APS Golden Jubilee volume, the Annual Reviews of Microbiology, of Plant Physiology, and of Entomology, Advances in Pest Control, and Botanical Review. To this list were added the chapter titles for the 3-volume treatise now being prepared for publication by Academic Press under the editorship of J. G. Horsfall, since they constitute review articles in essence.

Once a reasonably complete list of items already in progress was at hand, it became possible to issue invitations that would be little likely to duplicate material recently, or about to be, in print. Ten such invitations were sent out in the name of the committee in February, and necessary follow-up letters have been prepared from time to time since then. Six have committed themselves to prepare review articles, two tentatively agreed, one is still under consideration, and one individual has not replied. All in all, we believe this is at least as good a response as can be expected, for, important as may be the preparation of critical reviews, it is a laborious and little-honored task, and those who do it are making a considerable sacrifice for the benefit of their colleagues.

As an Advisory Editor to the Botanical Review, I have tried to see that no conflict whatever develops between the program of this committee and the publishing interests of the Review. At the time we issued ten invitations for

Phytopathology, I recommended ten titles to the Editor of the Review, Dr. Fulling. About half of these had been arranged for at the time of my last communication from Dr. Fulling.

Dr. Horsfall, as a special advisor to the board of the Annual Reviews of Plant Physiology, has been good enough to keep the committee informed of new titles being solicited for that journal. We thus hope to avoid duplication and waste.

Finally, during the past year I have had 6 of my students in plant pathology select titles from a provisional list of the kind supplied the Council at the 1957 meetings and assemble a provisional bibliography for the title chosen. They were then required to write brief summaries and, from their acquaintance with the literature, nominate a prospective author who might use their material as a starting point for a definitive review. These invitations will be issued shortly.

We received, several months ago, an unsolicited manuscript from Ernst Gäumann on the mechanism of action of fusaric acid. Because this was largely based on previously published material, it was turned over to the committee by Dr. Wilson with the request that we make recommendations for its disposal. After some correspondence and deliberation, the manuscript was returned to Dr. Wilson with the recommendation that it be accepted and published essentially in the form received, as the first in the review series.

Bibliography project. As authorized a year ago, the committee has prepared and submitted to the National Science Foundation a proposal for modest support of a project to assemble and prepare for publication a bibliography of reviews in plant pathology. As outlined, this effort would require the part-time services of a bibliographer and a typist for about a year and a half, and would look toward preparation of a bibliography covering English and foreign-language publications for perhaps a decade just past. We have in mind an annotated citation, and the grouping of titles into some logical pattern that could be used for subsequent entries as the program continues. The initial compilation will most probably appear as a special number of Phytopathology, and will then be followed by shorter lists, compiled each year along the same lines and using the same format. We have no way of knowing how large the initial list will be, but if the medical reviews bibliography is any measure, the task will be a substantial one. Our request to N.S.F. for $8,000 to prepare the bibliography of reviews has been approved, and the plans are to get started on the project by October 1, 1958.

The future. I see no need for major alterations in the Committee Program in 1959. It will be necessary to maintain constant pressure, without seeming to do so, if promised reviews are to materialize. It will be constantly necessary to seek new titles and to issue new invitations. It will be necessary to be ever vigilant in taking note of titles solicited and invitations accepted for reviews in journals other than Phytopathology, lest duplication ensue —and in many ways this is the most vexing problem we face. I think we have done well on this score to date, but it is a difficult thing to accomplish and must receive constant attention.

R. B. Stevens, *Chairman*

Report of the Committee on Placement. The applications of 53 plant pathologists were processed during the past year. Seventeen of these accepted employment and had their applications removed from the active files. Another 9 accepted new employment but desired to keep their application for placement on an active status. One other applicant requested withdrawal, although his employment did not change. There are now 35 applicants in the active files. Of these, 30 are U. S. citizens and 5 are not; 10 have had their applications with the Placement Committee for more than 2½ years.

During the year, 153 application records were sent to prospective employers, an average of 3 contacts (range of 0–8) for each applicant. On the other hand, 29 administrators were contacted regarding 38 positions. Application records from the Placement Committee files were considered for 29 of these positions, an average of 5 per position. Most of these positions have now been filled, though not necessarily through the contacts made by the Placement Committee.

In addition to those handled through the normal routine of the Placement Committee, 14 plant pathologists, who were employed by the Crops Division of the Biological Warfare Laboratories, Fort Detrick, Frederick, Maryland, found employment when this work was discontinued. Instead of using standard procedures of application blanks (2 exceptions), the Placement Committee prepared a mimeographed list of these men, with their degree, rating, and specialty, and mailed this to a large number of administrators who, if interested, were to contact the men directly. There is no record of the number of placements made through this service. Also, 3 recent graduates were placed by direct contacts made by committee members rather than through routine procedure.

An apparent weakness in the present operation of the Placement Committee is the low percentage of administrators who notify the Placement Committee of positions to be filled. With few exceptions, the committee learns of openings through local Department Heads or associates. This requires an exchange of letters before the Placement Committee is free to submit candidates for the position.

Persons interested in Federal positions should fill out Standard Form 57 and mail it to the Executive Secretary, Board of U. S. Civil Service Examiners, Sixth Floor, Administration Building, U. S. Department of Agriculture, Washington 25, D. C. On most Land Grant College campuses there is an Agricultural Research Service Contact Representative who can supply additional information or forms.

It is the responsibility of the applicants to keep the committee informed of changes in address or employment status. If there is no reply after 3 attempts to contact the applicant, his application is placed in the inactive file. All records and files are currently up-to-date.

W. E. Cooper, *Chairman*

Report of the Committee on Public Relations. This year our Committee, with the able assistance of Robert Rathbone, completed the brochure "Careers in Plant Pathology." Other work has continued along the lines initiated 4 years ago: publicizing some of the outstanding plant-disease research papers. Selected material appearing in Phytopathology has been used in advance, through the cooperation of the Editor-in-Chief, who made galleys available before publication. To achieve maximum effectiveness in this activity, we need more help from Society members. Authors should send us complete copies when their articles are accepted for publication elsewhere than in Phytopathology, since timing is most important in public relations work.

Emphasis this year has been placed on our Golden Anniversary. Plans for extensive news coverage, through the media of press, radio, and television, at the Bloomington meetings were coordinated with those of AIBS. It is hoped that certain outstanding symposium speakers and their papers can be featured. Press releases for about 20 contributed papers that seem to have publicity value will be issued at the time of presentation at Bloomington.

Paul R. Miller, *Chairman*

Report of the Committee on Regulatory Work and Foreign Plant Diseases. The Committee met in four sessions during the Palo Alto meetings of the Society, in August, 1957. It had invited the Committee on Seed and Plant Material Certification and the Advisory Committee on Nematology to meet jointly with it to consider some problems of mutual interest.

Six members of the Committee on Regulatory Work and Foreign Plant Diseases attended the first session, held

Sunday afternoon, August 25. Three members of the Committee on Seed and Plant Material Certification also attended, including Willis H. Wheeler, who was a member of both Committees. No members of the Advisory Committee on Nematology were present, but two nematologists attended. One was M. W. Allen, of the University of California at Berkeley, who had been requested by Harold J. Jensen of the Advisory Committee on Nematology to attend for him, since Jensen could not attend the Palo Alto meetings. The other was W. H. Hart, of the California Department of Agriculture, who had been invited by the Chairman of the Committee on Regulatory Work and Foreign Plant Diseases to discuss a problem of nematodes in imported primula seed. A second session, held the evening of August 25, was attended by 4 members of the Committee on Regulatory Work and Foreign Plant Diseases, including Wheeler. A third session, held Tuesday morning, August 27, was attended by the same 4 members, and also by 3 members of the Committee on Seed and Plant Material Certification, in addition to Wheeler. The fourth and final session, held on Wednesday morning, August 28, was attended by the same 4 members who attended the second and third sessions.

The following items were discussed and acted on by the Committee at the Palo Alto meetings:

ITEM 1. SPHERES OF COMMITTEE ACTIVITIES (first session). Discussion of this subject had been requested by the Chairman of the Committee on Seed and Plant Material Certification. It had seemed at times that there were areas of overlapping between that Committee and the Committee on Regulatory Work and Foreign Plant Diseases. After some discussion, the thought was expressed that the Committee on Seed and Plant Material Certification is concerned principally with problems of research on developing methods of finding, producing, and maintaining disease-free plant-propagating material for certification, whereas the Committee on Regulatory Work and Foreign Plant Diseases is concerned principally with matters of consultation and advice on plant quarantine and regulatory problems, especially with reference to the U. S. Department of Agriculture, the States, Commonwealths, and Territories. The latter Committee's sphere of activities, responsibilities, and methods of procedure have been defined by a Council resolution printed in the published report of the Committee on Regulatory Work and Foreign Plant Diseases in the Report of the Forty-seventh Annual Meeting of the American Phytopathological Society (Phytopathology 46: 352-353, 1956). It was suggested that the Committee on Seed and Plant Material Certification might submit, for Council approval, a resolution defining its sphere of activities, responsibilities, and methods of procedure, if it felt the need for more specific instruction and authorization from the Council.

The consensus at the joint session, after discussion, was that each Committee had separate and distinct fields in which to operate.

ITEM 2. NEMATODES AND SEEDS (first session). W. H. Hart related details on the recent finding of a form of the stem nematode, *Ditylenchus dipsaci* (Kuhn) Filipjev, causing serious damage to primula plants grown in California from seed imported from Italy. The instance was the first-known case of infection of primrose by *D. dipsaci* in California, and apparently was only the second report in the United States, the first having been made in a brief note by N. A. Cobb (Plant Disease Reporter 12: 10-11, 1928) on a specimen from Pennsylvania. Infection of primrose by this nematode is not uncommon in Europe. The propagation of primroses in California is not new, nor is the nematode species *D. dipsaci* new to the State. It was supposed, therefore, that the infection represented a *form* of the nematode new to California, not common in the United States, and, in this case, most likely of recent introduction. Investigation by the California Department of Agriculture resulted in tracing the infection to a lot of seed imported from Italy in which the nematodes were present between the seed coats. The U. S. Department of Agriculture, Plant Quarantine Division, was informed of the findings by the California officials, and the Department communicated with the Agricultural Attache at the American Embassy in Rome to bring the situation to the attention of the Italian Ministry of Agriculture for corrective measures.

There was further discussion on the problem of nematodes being carried in or with seeds. The Committee, at the Cincinnati meetings in December, 1956 (Phytopathology 48: 117-118, 1958), had recommended to the Society Council that the Society approve in principle and recommend that the U. S. Department of Agriculture consider the laboratory examination of imported seeds for the purpose of detecting seed-borne plant pathogens. The Society Council had concurred, and the Society's secretary had conveyed the recommendation to the U. S. Department of Agriculture through Dr. Byron T. Shaw, Administrator of the Agricultural Research Service.

It was the consensus of the group at the joint session that the Committee on Regulatory Work and Foreign Plant Diseases should request the A.P.S., through Secretary William B. Hewitt, to inquire of the U. S. Department of Agriculture as to developments since the recommendation of the Committee at the Cincinnati meetings. It was also suggested that the finding of the stem nematode in the imported primula seed from Italy be mentioned when making the inquiry as an example of the need for laboratory examination. The Committee voted to follow the suggestion and make the inquiry.

ITEM 3. INDEXING OF IMPORTED PRUNUS SPECIES FOR VIRUS DISEASES (first, third, and fourth sessions). The Interregional Technical Committee (now known as The Interregional Coordinating Committee) for Stone Fruit Virus Research, and the American Phytopathological Society's Committee on Regulatory Work and Foreign Plant Diseases, had recommended the establishment of a program for indexing imported plant materials for virus diseases. The Society, at the Atlanta meetings in December, 1955 (Phytopathology 46: 352-353, 1956), approved a resolution recommending the appropriation of funds by Congress to make possible the appointment of a plant pathologist to conduct the indexing. Funds had been provided, but there had been problems getting the program under way. In view of the expressed interest of the Society and of the interregional stone fruit virus research group in the matter, it was thought that the Committee should request the Society, through its Secretary, to inquire of the U. S. Department of Agriculture as to what progress has been made in the indexing program. The Committee instructed its chairman to make the request.

ITEM 4. RHODODENDRON-SPRUCE RUST PROBLEM (second session). Director E. P. Reagan, of the U. S. Department of Agriculture, Plant Quarantine Division, in a letter dated August 8, 1957, had referred to the Committee a problem concerning the European Rhododendron Rust, *Chrysomyxa ledi* var. *rhododendri*. The rust had appeared in two nurseries in the State of Washington, and the sale of plants from them had been restricted. It had not been found on native spruce, *Picea sitchensis* (Sitka spruce), in the region, although several species of *Picea* are known to be hosts for its aecidial stage. The rust is known to occur in Europe, Asia, New Zealand, and Canada, but not in the United States, aside from the two nurseries mentioned. According to the U. S. Forest Service, Division of Forest Disease Research, it has been found in Canada, on *Rhododendron lapponicum*, but isn't common there. It has not been found on spruce in North America; nor has the telial stage, which is necessary to initiate spruce infection, been observed on *Rhododendron* spp. on this continent. The Forest Service does not, on the basis of present information, consider that this rust is a threat to *Picea* species.

In addition to the restrictions placed on the two affected Washington nurseries, federal post-entry quarantine requirements had been placed on rhododendrons because of this rust fungus.

The U. S. Department of Agriculture, Plant Quarantine Division, requested the Committee's recommendation on the following: 1) should the restrictions on the movement of possible infected plants be removed; 2) should the post-entry quarantine requirements on rhododendrons be removed, thus making it unnecessary for the importer to hold them about two years in a designated place for field examination? It appeared to the Committee that spread of the rust among rhododendron plants through urediospores, as well as among other uredial stage hosts, must be taken into consideration along with possible spread to spruce, even though spread to spruce has not yet been apparent in North America. It was the opinion of the Committee that the biologically sound course is to continue to contain the infection where it exists in the State of Washington, to eradicate the infection if possible, and, in the meantime, to continue the post-entry quarantine protection. The Committee instructed its chairman to convey this opinion to Director Reagan.

ITEM 5. PHOMOPSIS DISEASE OF CONIFERS (second session). For many years the U. S. Department of Agriculture has prohibited importation from Europe of the known hosts of the causal fungus of the Phomopsis disease of conifers, originally described by Malcolm Wilson as *Phomopsis pseudotsugae* (Bot. Soc. Edin. Trans. 28: 47-49, 1920). Glenn Gardner Hahn discussed the fungus recently in an article entitled "A New Species of Phacidiella Causing the So-Called Phomopsis Disease of Conifers" (Mycologia 49: 226-239, 1957), and also in an article entitled "Phacidiopycnis (Phomopsis) Canker and Dieback of Conifers" (Plant Disease Reporter 41: 623-633, 1957). Now that Hahn has shown this fungus to be present in the United States, where it apparently has been for many years and where it may be native, and since no restrictions because of this fungus are placed on the movement of host trees in this country, the U. S. Department of Agriculture, Plant Quarantine Division, wondered whether the federal prohibition on hosts of this fungus from Europe was quite consistent with this more recent information. It was the thinking of the Committee that the Plant Quarantine Division should explore the matter further with the individual states.

ITEM 6. POST-ENTRY QUARANTINE REQUIREMENTS FOR IMPORTED VEGETATIVELY PROPAGATED EAST MALLING UNDERSTOCKS (second session). The U. S. Department of Agriculture had been requiring post-entry quarantine for vegetatively propagated understocks entering the United States from Europe. The principal concern had been with apple understocks in relation to brown rot disease caused by the fungus *Monilinia fructigena*. The Department had been asking that the original imported stocks not be budded or grafted during post-entry quarantine observation and that, during this period, they be grown in stool beds, where they would not bloom or fruit, and wherein more material could be propagated for budding while the understocks were under observation for the brown rot fungus and other fungus and bacterial pathogens.

During the years since 1948, when Federal Quarantine No. 37 was revised, no evidence had been found of an introduced fungus or bacterial disease on the imported East Malling stocks. In the meantime, more and more knowledge had come, in general, on virus diseases, but no virus diseases had been detected during field inspections of the stool beds. The Department was considering removal of the stool-bed requirement and permitting the budding of the imported understocks during the first year of post-entry quarantine. Such a program would permit seeing the apple understocks in growth the first growing season, and seeing the growth from the bud or graft during the second growing season. This plan is being followed with rose stocks.

The Committee saw no objection to the Department's proposal. It appeared to the Committee that inspection of the growth from the variety bud might give an added opportunity to detect the presence of virus infection, because the understock could be a symptomless carrier while the apple variety budded onto it might be a better virus indicator and show symptoms that could not be detected in two years' growth of the understock.

FEDERAL PLANT PEST ACT. The legislation proposed in Senate Bill No. S3952 of the 84th Congress, which had been approved in principle by the Committee and by the Society and which had failed to become enacted into law before the Congress adjourned, was resubmitted in a new bill to the next Congress, where it passed and became law (Public Law 85-36, 85th Congress, S. 1442, May 23, 1957).

GILBERT L. STOUT, *Chairman*

Report of the Committee on Seed and Plant Material Certification. Because of lack of information, some American seed exporters apparently have failed to comply with the importation certification requirements of the country of destination. A memorandum was prepared and sent to trade papers and seed associations, indicating that the necessary specific information was available on request from the Plant Quarantine Division, U. S. Department of Agriculture, Washington 25, D. C.

The Committee has been in touch with the International Seed Testing Association's Committee on Plant Diseases. They are proceeding with the publication of brief monographs on diseases caused by seed-borne pathogens. Specialists who are willing to prepare short statements on individual diseases of this type are urged to get in touch with them through this Committee.

K. F. BAKER, *Chairman*

Report of the Committee on Fungicide Colloquium. The recommendation was made, in the last Annual Report, that the Fungicide Colloquium be suspended for 1958 in deference to the nature and scope of the 50th-Anniversary Program of the Society.

The Committee has provided comments and suggestions concerning the Anniversary Program, and some of its members have participated in more tangible ways.

The Colloquium, over the past several years, has acquired both recognition and status as an integral and important part of the annual programs of the Society. The Committee, therefore, recommends that it be continued and that definite plans be made for the Fungicide Colloquium to be included on the program of the Annual Meeting of the Society in 1959.

L. GORDON UTTER, *Chairman*

Report of the Committee on Industrial Relations. During the past year the main activity of the Industrial Relations Committee has been to secure the financial support from industry for the Golden Jubilee Celebration of the Society. There were 102 Industrial concerns contacted. Although $15,000 was set as a goal for industry, the total contributions secured to date are $9,625. The difficulty in obtaining more funds has been largely due to the general recession during the past 8–10 months.

Various industrial organizations that in the past would be expected to give strong support, either refused or made only small contributions. Most of these organizations were contacted by individual members of the committee after the official letter signed by the chairman was received.

A few organizations are still being contacted. With the current upturn in business, there may still be a chance of additional responses, although this is very doubtful.

E. F. FEICHTMEIR, *Chairman*

Report of the Advisory Committee on Nematology. No special problems were brought to our attention for consideration during the past year. The advisory committee was represented at a meeting of the Standing Committee on Regulatory Work and Foreign Plant Disease, A.P.S. at Stanford University, Palo Alto, California, August 25–28, 1957, by Dr. M. W. Allen.

The advisory committee strongly endorses the stand taken by the committee on Regulatory Work and Foreign Plant Diseases, A.P.S., on the great importance of labora-

tory examination of imported seeds for detection of seed-borne plant pathogens including nematodes.

Possible future activities of the advisory committee are as follows: a) Publishing an approved list of common names for nematodes, which would serve as the approved list for publishing in PHYTOPATHOLOGY. b) Reporting results of new nematocides similar to the fungicide tests. c) Publishing a list of all members actively engaged in nematological work (location, major interest, etc.). d) Selecting an outstanding paper to be submitted to the committee on Phytopathological Classics. It is appropriate that nematology be represented in the series.

J. N. SASSER, *Chairman*

Report of the Committee on Membership. During the past year the Membership Committee made a concerted drive for new members, with emphasis on graduate students. In cooperation with S. E. A. McCallan, the drive was built around the advantages of being listed in the new Directory of Members, and graduate students were offered the additional inducement of the $5.00 dues.

Working against the publication deadline of the new Directory, two drives were made: one in October to catch the crop of new graduate students, and a second in December, about a month before the Directory was to go to press.

Members of the Committee cooperated fully in these drives. The United States and Canada were organized in sections, and each section was the responsibility of a Committee member. Since the Committee does not keep figures on recruitments, it has no measure of its effectiveness.

WILLIAM C. SNYDER, *Chairman*

Report of the Committee on Teaching of Plant Pathology. Publication of the pamphlet "Career Opportunities in Plant Pathology," in cooperation with the Public Relations Committee, completed an undertaking begun about five years ago. About 500 copies were mailed to each department of plant pathology for distribution to high schools. A few departments requested an additional supply, and their requests were filled. Accumulated requests from guidance officers, teachers, and individuals totaling 50 were also filled. Opportunities developed for the chairman to distribute 200 copies of the pamphlet to National Science Foundation Institutes at the South Dakota State College, Brookings, the University of South Dakota, Vermillion, and the Iowa State College, Ames. Of the 50,000 printed, 7350 are held for distribution by the chairman at Ames. Dr. Paul Miller has 3000 copies to answer requests coming to his office.

An unusual opportunity exists in the form of the National Science Foundation Institutes for getting the pamphlets into the hands of science teachers. About 300 Institutes are planned for next summer (1959), and the chairman recommends distributing 75 copies to each, a total of 22,500.

Is the pamphlet satisfactory, or should plans for a more complete treatment on "slick" paper (10–20 pages), copiously illustrated and costing $3000–$4000 for 10,000–20,000 copies be a next order of business? Such a publication, discussed and approved at last year's Council meeting, could be distributed to high school and college counselors and filed in libraries. Other professional societies have followed such a procedure. Teachers, students, and the public generally are not adequately acquainted with the science of plant pathology, so more needs to be done. The chairman recommends preparation of a more complete treatment.

Several departments have initiated undergraduate curricula in plant pathology during the last few years. Demands for more intensive and extensive training in the physical sciences and mathematics are being made by some employers. To what extent are these developments indicative of significant trends in training plant pathologists? Should the Committee on Teaching plan a symposium on "Training Plant Pathologists" for next year's meetings? Since nothing along this line has been discussed before the society in recent years, and since many departments have changed leadership in the interim, it is the recommendation of the chairman that the Committee be permitted to organize such a symposium in 1959 or 1960.

W. H. BRAGONIER, *Chairman*

Report of the Special Committee on Utilization of Plant Pathologists and Facilities in National Emergencies. The committee had little activity, since only one request for aid in obtaining an appropriate military assignment was requested. In connection with this request, the committee contacted the office of the Adjutant General of the Army and obtained a prompt and thorough analysis of the situation, i.e., enlistment as a critically skilled specialist that would require only 3 months on military active duty with an assignment at Fort Dix, where, if possible, his civilian experience would be utilized. The committee recommends that this special committee be continued for 1959.

H. H. THORNBERRY, *Chairman*

Report of the Committee on Virus Type Culture Collection. Revision of the Catalogue and Registry of the Plant Viruses is near completion, and the card records soon will be ready for typing in manuscript.

Work on the cooperative virus antiserum project continues. The purified antigens were prepared by the collaborators and mailed to the Microbiological Research Foundation, Bethesda, Md. The Foundation conducted all of the animal work. The sera were collected at intervals following the initial injection, mailed to Lincoln, Nebraska, and titrated for antibody content in the virus laboratory of the Department of Plant Pathology, Nebraska Agricultural Experiment Station, in cooperation with the Crops Research Division, U. S. Department of Agriculture.

Pooling the final antisera is now in process, and samples are being sent to Lincoln for titration. Samples will be sent to each of the collaborators and to other qualified persons for evaluation. As soon as the results are assembled, a full report will be prepared for publication.

Viruses for which antisera are prepared	*Contributor*
Barley stripe mosaic	M. K. Brakke
Brome grass mosaic	M. K. Brakke
Cymbidium mosaic (non-specific agglut.)	A. H. Gold
Potato latent mosaic (X virus)	A. F. Ross
Tobacco mosaic	H. H. Thornberry
Tobacco ringspot	R. L. Steere

Antisera for alfalfa mosaic virus contributed by R. L. Steere, and for southern bean mosaic virus contributed by M. K. Brakke, are still in the process of preparation.

H. H. MCKINNEY, *Chairman*

Report of the Advisory Committee on Collecting and Disseminating Data on New Fungicide Tests. The Council, in accordance with the recommendations of the Committee, approved the appointment of Dr. A. B. Groves, Department of Plant Pathology, Virginia Experiment Station, Winchester, Virginia, to become coordinator of the project. Dr. Groves has assumed all responsibility in soliciting and preparing the data and manuscript for publication of the "Results of the 1957 Fungicide Tests." He also administered the handling and filling of orders, and others matters related to efficient handling of the entire project.

In our 1956–1957 Annual Report it was indicated that *Agricultural Chemicals* might not find it possible to continue publication of the "Report," because of the increased number of pages, tabular material, and printing costs. This situation became a fact, and careful consideration was given by the Committee to other magazines or journals. Such media, however, appeared to be confronted with similar difficulties with respect to such manuscripts as the "Report." Cost estimates were obtained from various printers for direct printing of the "Report." Dr. Groves was able to have the manuscript printed, with covers, at a cost of $375.20 for 1200 copies, which was the best figure

offered. This amounts to $.3127 per copy, compared to $.163 per copy for the 1956 Report. It should be pointed out, however, that the 1957 Report contains 44 pages, compared to 24 pages for the 1956 Report. Furthermore, by publishing the Report in this manner it was possible to distribute copies around the first of March instead of later, as in past years. This immediately enhanced the value of the "Report" for use during the current season. In addition, availability of the "Report" only through purchase has changed the demand for copies from that of previous years.

Dr. Groves has initiated some changes of value in the "Results of the 1957 Fungicide Tests." These include preserving the identity of the separate reports submitted, alphabetical indexing by state or province, grouping the reports received by field of crop interest in final compilation and results summarized by materials tested, and providing a section on newer materials available for testing. Copies of the "Report" were sent each cooperator.

Publicity on the availability of the "Report" was provided through the fine cooperation of *Plant Disease Reporter* and *Agricultural Chemicals*. Notices also appeared in *Croplife*, *Science*, *Journal of Economic Entomology*, *Journal of Agricultural and Food Chemistry*, *A.I.B.S. Bulletin*, *Better Farming Methods*, *American Potato Journal*, *American Fruit Grower*, *Crops and Soils*, *NAC News*, and *Phytopathology*.

Because of transference of the project from Cornell to Dr. Groves, a detailed report on the final status of the 1956 Report can be provided only as follows:

Received from Sale of 424 copies	$424.25
Accounts due on 1956 Sales	6.60
Total	$430.85

Dr. Groves, as of July 11, 1958, summarized the status of the 1957 Report as follows:

Received from Sale of 693 copies	$649.30
Accounts due on 1957 Sales	55.30
Total	$704.60
Expenditures on publication:	
Printing 1200 copies	375.20
Stenographic and Secretarial:	
Miss Ferguson	200.00
Miss Parker	18.00
Stationery and Supplies	60.30
Postage and Postal Cards	24.93
	$678.43

This indicates a profit of $26.17. It is anticipated that an additional 50 or 60 copies of the Report will be sold in the near future, thereby increasing the profit for 1957. However, no remuneration has been deducted by Dr. Groves, which the Committee considers he is entitled to for his time and efforts on the project. It appears that the project is self-supporting on the basis of present expenditures.

A recapitulation of the financial status of the project based on the previous year's report and Dr. Groves' statement of July 11, 1958 shows the following:

August 1957:	
Total receipts	$2,300.00
Total sales	1,441.85
Total Available	$3,741.85
Total expenditures	2,453.25
Balance on Hand	$1,288.60
Expenditures—August–December 1957	309.26
Fund Transferred (Dr. Rich to Dr. Groves—12/23/57)	$ 979.34
July 1957 Statement:	
Deposits	875.64
Total Available	$1,854.98
Total expenditures	505.40
Balance on Hand	$1,349.58

Petty cash was reported at $20.14

The balance of $1,349.58 shows an improvement over the corresponding balance of August, 1957. The fund of $2,300, originally subscribed to initiate this project, has been depleted by $950.42 to underwrite losses, as indicated, from previous "Reports." The present status of the "Report" would indicate that a self-supporting basis is being reached, which it is hoped can be attained in the future in confirmation of the original optimism of the Committee.

L. Gordon Utter, *Chairman*

Report of the Advisory Committee on Integration of Research on Soil-Borne Pathogens. In its second year this Committee continued to direct its major attention toward bringing together those investigators who are concerned directly with soil-borne pathogens.

During the year the Committee fostered the reciprocal interchange of technical committee members of the Southern and Western Regional Research Projects. Furthermore, it encouraged broad intra-regional as well as inter-regional representation at these meetings, and the inclusion of foreign visitors when possible. The Committee has considered that this sort of activity was one of the primary reasons for its creation. At these meetings, such as those held at Clemson, South Carolina, and Corvallis, Oregon, the broad national representation present contributed greatly to the interest and scope of the meetings, stimulated discussion of common problems, and engendered an international outlook. The enthusiasm and response has been very rewarding.

Other activities of the Committee included unofficial participation in the selection of the panel of speakers for the Symposium on Soil Microbiology and Root Disease Fungi, held at this year's meeting, and in the encouragement of national and international representation at the Pacific Coast Conference on the Control of Soil-Borne Fungi.

In spite of the widely separated locations of the Committee members, the Committee managed to hold one meeting this year. Attention there was given to the problem of exchanging information on new techniques for dealing with soil-borne organisms, since these seem to be developing faster than they are being published. In this connection the Committee strongly endorsed the activities of Dr. Leander F. Johnson and associates in the completion of their project along these lines, and this has been essentially accomplished.

The Committee also has given considerable thought to the problem of how best to make available quickly, and possibly in abstract form, world literature in this area of research to all those actively investigating soil-borne organisms. Dr. L. W. Koch and his associates have been looking into this problem.

As chairman, the question was raised with the Committee members as to whether the purpose of the Committee had now been served and whether the recommendation should be made to the Council that the Committee be disbanded. Committee members responded rather strongly in favor of retaining the Committee. They pointed out that interest in this area of plant-pathological research is finally growing rapidly in the United States, and that this is the time when an official committee may be most effective in encouraging such research and in exchanging techniques, information, and personnel.

In view of this, the Council may wish to consider extending the life of the Committee another year, and possibly increasing its membership.

William C. Snyder, *Chairman*

Report of the Fungicide Advisory Committee. Only one matter has been brought to our attention. It was suggested that the question be considered of having fungicide reference standards prepared in a manner similar to that of the Entomological Society of America. These standardized fungicides would serve as references in research, bioassays, and similar laboratory studies. The E.S.A. insecticide standards are packaged and sold by Nutritional Biochemicals Corporation. The background work, choice of standard insecticides, and so on is done by a special committee of the E.S.A. The source of the chemicals is a selected primary manufacturer.

Three members of the committee were opposed to the idea, two had no marked opinions, and one was in favor. It is the consensus that demand for standard reference fungicides is presently insufficient to warrant a recommendation that The American Phytopathological Society engage in this activity, which would demand time and expense.

S. E. A. McCallan, *Chairman*

Report of the Fiftieth-Anniversary Committee. The Anniversary Committee as appointed by the Council organized itself into a number of Subcommittees and enlisted the help of many additional persons. The entire organization was as follows:

General Committee—Glenn S. Pound, Chr.; Wayne Bever, D. E. Ellis, J. G. Harrar, Wm. B. Hewitt, C. S. Holton, H. A. Rodenhiser, J. R. Shay, R. H. Wellman.

Subcommittees

Symposia:

Viruses—Glenn S. Pound, Chr.; L. M. Black, A. F. Ross
Fungicides—R. H. Wellman, Chr.; A. E. Dimond, R. A. Ludwig
Physiology of Parasitism—J. R. Shay, Chr.; Paul Allen, Stephen Wilhelm
Pathogenicity of Fungi—H. A. Rodenhiser, Chr.; J. J. Christensen, G. W. Keitt
Soil Microbiology—Wm. B. Hewitt, Chr.; W. C. Snyder, N. C. Thornton
Nematology—D. E. Ellis, Chr.; D. J. Raski, A. L. Taylor
Epidemiology—Wayne Bever, Chr.; Paul R. Miller, Paul Waggoner

Plant Pathology Moving Picture Film—J. R. Shay, Chr.; Gordon Brandes, Eugene Hayden, George Kent, Howard Lyons

Exhibits

Planning—S. E. A. McCallan, Chr.; R. S. Kirby, Charles Krister, Curt Leben, R. H. Wellman
Setup—Ralph J. Green, Jr., Chr.; Curt Leben

Publicity—Paul R. Miller, Chr.; Gordon Brandes, S. J. P. Chilton, E. F. Feichtmeir, Don Fletcher, R. J. Haskell, Eugene Hayden, Paul Hoffman, R. S. Kirby, H. Rex Thomas, R. H. Wellman

Local Arrangements—J. R. Shay, Chr.; C. C. Allison, W. M. Bever, Stephen Diachun, R. J. Green, Jr., Curt Leben

Industrial Relations—E. F. Feichtmeir, Chr.; Paul Hoffman, C. J. Krister, G. L. McNew, N. C. Thornton, J. S. Tidd, L. G. Utter, R. H. Wellman, H. C. Young, Roy A. Young, S. G. Younkin

Golden Jubilee Volume of Reviews

Editorial—C. S. Holton, Chr.; George Fischer, R. W. Fulton, Helen Hart, S. E. A. McCallan
Publicity and Sales—George A. Zentmyer, Chr.; J. F. L. Childs, C. S. Holton, G. C. Kent, W. D. McClellan, G. S. Pound, Saul Rich, R. H. Wellman

Anniversary Banquet.—J. H. Jensen, Chr.; W. H. Bragonier, T. H. King, T. C. Ryker, J. M. Wallace

A) *Symposia.* Nine symposia and 6 major addresses dealing with historical and developmental aspects of plant pathology constituted the core of our anniversary program. These were well attended, and subject presentations were well received. These were all recorded on tape for historical records.

B) *Speakers Bureau.* Arrangements were made through A.I.B.S. for support of a speakers' bureau whereby 4–5 of our members were invited to make 4–5 appearances before college audiences.

C) *Film.* A 16-mm color-sound movie is being made. This film is being designed for adult (including H.S. youth) audiences and deals with the "what" and "why" of plant pathology. Much deliberation was given to the message the film would carry. It was finally decided that it should acquaint the lay public with what plant pathologists do and why. It will lay the groundwork for more special films to follow, if such can be produced. It will be ready in the late fall of 1958.

D) *Exhibits.* An elaborate series of exhibits dealing with a variety of subject matter were displayed. These were prepared by individual institutions at their own expense, but APS paid for booth rentals, services, etc.

E) *Anniversary Banquet.* This was truly one of the highlights of the celebration. The size of the banquet hall limited attendance to 900, and many were turned away. It was at this program that official recognition was made of visiting delegates.

F) *Publication of Symposium Papers.* The symposium papers are to be in a special volume, "Plant Pathology—Progress and Problems, 1908–1958," published by the University of Wisconsin Press. It should be available in early 1959.

G) *Finances.* It is a pleasure to acknowledge financial support from a number of sources. Travel and travel funds were supplied by the Rockefeller Foundation, The National Science Foundation, The Office of Naval Research, and individual institutions. The film is being paid for in large part with contributions from Industrial contributions. The publication costs of the volume of symposium papers was underwritten by the National Science Foundation. Our own membership appropriated $15,000 toward the meeting.

H) *Attendance.* It is estimated that about 1200 of our members registered for the meetings. Some 50 official delegates represented other American societies and institutions, and societies in other countries.

The Anniversary Committee is deeply indebted to many persons and institutions for bringing success to our program. Many people have been at work, and many contacts have been made. Plant pathology is on a firm footing from which to step out into the next 50 years.

Glenn S. Pound, *Chairman*

Report of the Resolutions Committee. *WHEREAS* the American Phytopathological Society has been celebrating the golden moments of its fiftieth birthday, and now, like many of its members, goes on to the silver, and

Whereas this memorable occasion has been so pleasantly and comfortably accommodated in the venerable modernity of the lovely and serene atmosphere of the campus of the Indiana University, and,

Whereas the local arrangements have been so thoughtfully arranged, and have functioned with such quiet smoothness, that the participants have been able to wrestle with ideas instead of physical survival, and have grown word-weary more pleasantly and less exhaustingly than ever before, and,

Whereas the weather was so perfectly controlled that rains just before the Celebration scrubbed the air of spore showers and academic stuffiness, and presumably after the meetings will wash away the debris of abandoned committee reports, discarded resolutions, and shattered scientific theories, but maintained crisp, sunny weather during our deliberations, and,

Whereas the keynote speech fittingly explained how plant pathology passed through the spore stage of emphasis on the ancient trinity of air, water, and fire, through the vegetative stages of predisposition, mycogenesis, and pathogenesis, to the present more perfect form, when it is truly up in the air, and,

Whereas we have been regaled by an account of how one famous participant shared his bride with a head of

cabbage, how another became so bemused as to think he was hearing fairy tales, and of how Albemarle crossed a bridge before he came to it, and,

WHEREAS the unfortunate attempt in the new Society Directory to exaggerate the pathological California colossus was finally set aright, only to be revived by their domination in the presentation of unwanted contributed papers, and,

WHEREAS more members have participated in this meeting than ever before, many of whom found that it is very difficult to depart from this Lorelei Rock,

THEREFORE BE IT RESOLVED that the American Phytopathological Society expresses its deep appreciation of the dedicated service of the following, who did so much to ensure the success of the celebration:

The General Committee of the Society, so ably led by Glenn Pound.

Indiana University, for being the perfect host to a group of imperfect parasites, for providing suitable infestation courts, optimal nutrition, and favorable environmental conditions for symptom expression. Particularly appreciated is the assistance of President Herman B. Wells, Norris Wentworth, Chairman of the University Educational Conference Bureau, and David G. Frey, Chairman of the University Faculty Committee for the A.I.B.S. Meetings.

The inspired cooperation of Hiden T. Cox and Ileen E. Stewart of the A.I.B.S.

The Local Arrangements Committee of our Society, so ably chairmanned by Ralph Shay, and the projectionists and electricians who so kindly maintained the darkness conducive to recuperation from the exhaustive discussions.

The Committee on Industrial Relations, led by E. F. Feichtmeir, and the many contributors who financially made possible this unprecedented undertaking.

The Symposia subcommittees and the participating speakers who presented such an admirable summary of Plant Pathology in the year 1958.

The Exhibit Committee, headed by S. E. A. McCallan, for arranging the excellent visual evidence of Fifty Years of Progress, and for calling attention to the handsome captains who must guide the Phytopathological fleet into the lush green of a better fiscal era.

The Anniversary Banquet Committee, headed and toasted by J. H. Jensen, amply supported by heavy artillery.

The Golden Jubilee Volume so competently being edited by a group of journalists headed by C. S. Holton, and sold (we hope) by a group of salesmen managed by G. A. Zentmyer.

The Publicity Committee, headed by Paul R. Miller.

The American Phytopathological Society takes pleasure in acknowledging that seldom have so many owed so much to so few. Thus spake Zarathustra—for the fourth time.

K. F. BAKER
C. J. NUSBAUM
E. L. KENDRICK

REPORT OF ELECTIONS, AND GENERAL BUSINESS OF THE ANNUAL MEETING AND OF THE COUNCIL. The ballots of the annual election were counted by a special committee appointed by President Paul Miller and approved by the Council. The following were elected by a majority of votes cast: President Elect: W. C. Snyder; Vice-President: S. E. A. McCallan; and Councilor-at-Large: Ralph Shay.

At the general business meeting the Society approved changes in the Constitution, Article V, Section 2, to read as follows: Section 2. The Secretary and the Treasurer shall be appointed by the Council, ordinarily for terms of three years, which shall not expire concurrently, and the Council may adjust the term or the date of assuming office to avoid this contingency. Individuals shall not serve more than two terms consecutively.

The Society approved changes in Standing Rule 2 (c) to read as follows: (c) *Terms of Office.* The term of each member of the Board shall be three years, and the appointments, exclusive of that of Editor-in-Chief, shall be staggered so that five members are appointed each year. The Council is authorized to make any adjustment in these terms of office necessary to effect non-concurrence. Individuals appointed to the office of Editor-in-Chief or Business Manager shall not serve more than two terms consecutively.

The Society approved the recommendations of the Council appointing the representatives, Editors of PHYTOPATHOLOGY, and Committees listed earlier in this report. The Society also approved the following recommendations of the Council:

1. That the Society again waive the $1,000.00 membership fee to the A.I.B.S. and pay $1.00 per member again this year.

2. That the Society contribute $200.00 to the American Type Culture Collection to help defray the costs of publishing a revised edition of the plant virus catalogue.

3. That the Society continue a representative on the American Grassland Council (formerly the Joint Committee for Grassland Farming) and pay the annual dues of $10.00.

4. That the Society again provide, as it has in the past few years, a $1500.00 fund for the use of the Committee on Public Relations. (Note that the Committee has not used the full amount but needs to have this amount available to assure ample latitude.)

5. That the Committee on Public Relations be authorized to reprint the pamphlet "Careers in Plant Pathology," should the need arise within the coming year, and that the Society provide $2500.00 for the costs of reprinting the pamphlet. (*Note:* this will provide a printing of 100,000 copies, and it is understood that the committee will bring sections up to date where needed.)

6. That a committee be appointed to study the possibility of establishing a facility or center not only for the collection, maintenance, and distribution, but also for the study of fungi and other microorganisms, and that the committee report back their findings and recommendations to the Council by next year.

7. That the Society form an "Advisory Committee in Agricultural Meteorology" to serve in a liaison capacity between the American Phytopathological Society and the American Meteorological Society. They recommend the appointment of: Paul Waggoner, Chairman; S. M. Pady, J. R. Wallin, R. A. Hyre, R. V. Bega, E. P. Van Arsdel, L. H. Person.

FUTURE MEETINGS. The Council confirmed the following meetings: 1959—Pennsylvania State University, University Park, Pennsylvania, August 30–September 2, in conjunction with the A.I.B.S. 1960—The American Baptist Assembly, Green Lake, Wisconsin, August 28–31, in conjunction with the Potato Association of America.